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STUDIES ON LYMPHOID ACTIVITY.

V. Relation between the Time and Extent of Lymphoid Stimulation Induced by Physical Agents and the Degree of Resistance to Cancer in Mice.

By JAMES B. MURPHY, M.D., WARO NAKAHARA, Ph.D., and ERNEST STURM.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, December 31, 1920.)

Two methods have been described by which a definite stimulation of the circulating lymphocytes, accompanied by a hyperactivity of the lymphoid centers, may be brought about. It was first noted that small doses of x-rays would induce this condition,[1] but the reaction was of short duration[2] as compared with the stimulation occurring in cancer-immune mice following inoculation of cancer.[3] The stimulation induced by x-rays is preceded by a period during which evidences of the destructive action of this agent on the lymphoid centers are present, and is followed by a period during which the stimulation phase alone is present. By the 4th day the proliferative activity is at its height and then quickly subsides.

The amount of stimulus produced by dry heat[4] is much greater in extent and of longer duration than that seen after exposure to x-rays. Immediately after the heat application there are also marked evidences of cell destruction in the lymphoid centers, but the stimulation following is more prompt and of greater volume as judged by the blood pictures.

[1] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800. Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 75.

[2] Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1920, xxxi, 13.

[3] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204. Murphy, Jas. B., and Nakahara, W., *J. Exp. Med.*, 1920, xxxi, 1.

[4] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1.

We have described the close relation existing between lymphoid stimulation and the resistant state to cancer inoculation. If this relation is quantitative in nature, it would be expected that mice whose lymphoid cells are stimulated by x-rays would show a definite degree of resistance but less than that exhibited by animals following heat stimulation. This fact is borne out by our figures on 102 mice heated 1 week before inoculation, which showed an average immunity of 60.3 per cent, controlled by 83 normal mice inoculated with the same tumors showing an average immunity of 16.5 per cent. On the other hand, 144 mice, x-rayed from 3 to 7 days before inoculation, had an immunity of 37.5 per cent; they were controlled by 137 normal mice inoculated with the same tumors, having an average immunity of 10.4 per cent.

If the hypothesis is true that stimulation of the lymphocytes of mice definitely reduces the number of takes of cancer grafts, it would be of importance to know whether there is a difference in resistance percentage when the tumor inoculation is made at varying times after the stimulus is administered. The following experiments were planned to test this point.[5]

Immunity after Exposure to X-Rays.

Mice of about the same age were divided into three lots. Two of these lots were given a dose of x-rays governed by the following factors: spark-gap $\frac{3}{8}$ inch between points, milliamperage 25, distance from target to back of animals 8 inches, and time 10 minutes. One lot received this dose 7 days and the other immediately before they were inoculated with a tumor (Bashford 63). At the same time the third group was inoculated with the same material and weekly measurements were made of the resultant tumors.

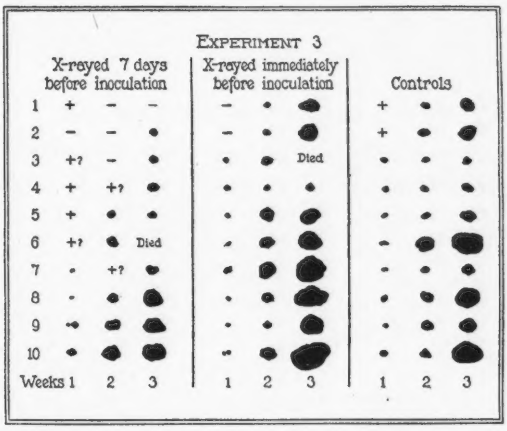
Table I gives the results of six experiments, the percentage of immune mice being estimated for a period 3 weeks after inoculation (Text-figs. 1 and 2).

[5] A preliminary report of this work was made before the National Academy of Science (Murphy, Jas. B., *Proc. Nat. Acad. Sc.*, 1920, vi, 35).

TABLE I.

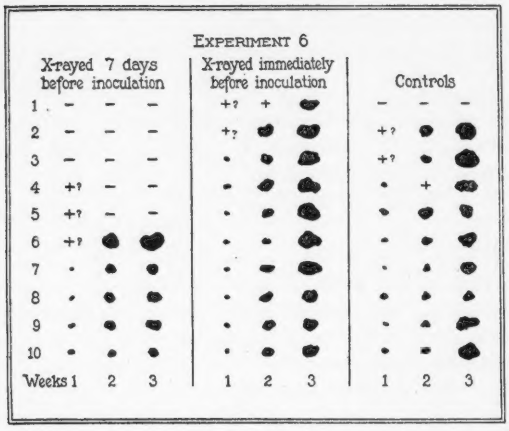
Experiment No.	Group 1.	Group 2.	Group 3.
1	11.1 per cent (9 mice).	0.0 per cent (10 mice).	0.0 per cent (10 mice).
2	30.0 " " (10 ").	12.5 " " (8 ").	10.0 " " (10 ").
3	10.0 " " (10 ").	0.0 " " (10 ").	0.0 " " (10 ").
4	50.0 " " (10 ").	10.0 " " (10 ").	20.0 " " (10 ").
5	40.0 " " (10 ").	11.1 " " (9 ").	11.1 " " (9 ").
6	50.0 " " (10 ").	0.0 " " (10 ").	10.0 " " (10 ").

Group 1 was made of mice given a dose of x-rays 7 days before cancer was inoculated. Group 2 animals were x-rayed immediately before the cancer inoculation. Group 3 comprised the control mice inoculated with the same cancers. 175 mice were used for these tests.



TEXT-FIG. 1. The effect of x-rays on the immunity to transplanted cancer when administered 7 days and immediately before inoculation.

From the foregoing experiments it is seen that the resistance of mice inoculated with cancer immediately after a stimulating dose of x-rays is no higher than that of normal mice, and on the average is somewhat lower. On the other hand, the mice inoculated with cancer a week after a stimulating dose of x-rays show a consistently higher degree of resistance which reveals itself both in the number of takes and in the rate of growth of the tumor.



TEXT-FIG. 2. The effect of x-rays on the immunity to transplanted cancer when administered 7 days and immediately before inoculation.

Immunity after Exposure to Dry Heat.

These experiments with x-rays have been paralleled with similar tests with heat as the agent for stimulating the lymphocytes.

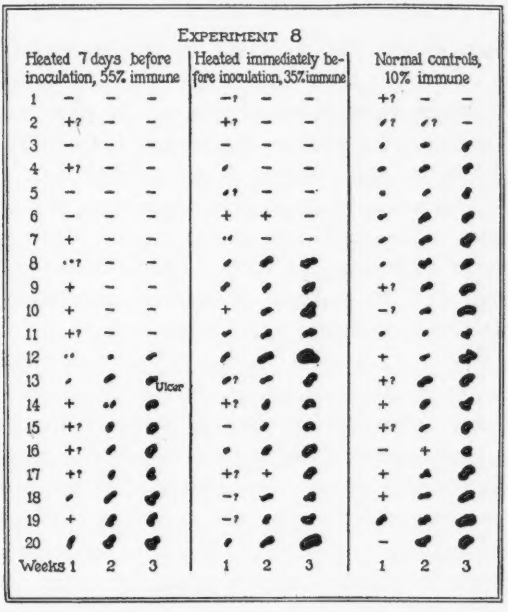
Three groups of mice were used. Group 1 consisted of animals which had been heated 1 week previous to the inoculation with cancer. Group 2 mice were heated immediately before the cancer inoculation, and Group 3 consisted of the normal controls inoculated with cancer. The mice to be heated were placed in an enclosed cage 3 inches above an electric heating lamp. A thermometer was placed half an inch below the bottom of the cage. The temperature was allowed to rise to 55°C. before the animals were put in the cage and then they were left in for 5 minutes, the temperature rarely rising above 63°C. during this time.

The results of the three experiments are given in Table II, the figures representing the immunity percentage 3 weeks after inoculation with cancer (Text-fig. 3).

TABLE II.

Experiment No.	Group 1.	Group 2.	Group 3.
7	66.6 per cent immunity.	40.0 per cent immunity.	20.0 per cent immunity.
8	55.0 " " "	35.0 " " "	10.0 " " "
9	50.0 " " "	30.0 " " "	10.0 " " "

Group 1 was made up of mice heated 1 week before inoculation. Group 2 mice were heated immediately before inoculation, and Group 3 comprised the control mice. The results are based on 118 mice.


EXPERIMENT 8
Heated 7 days before inoculation, 55% immune
Heated immediately before inoculation, 35% immune
Normal controls, 10% immune
Ulcer
Weeks 1 2 3 1 2 3 1 2 3

TEXT-FIG. 3. The effect of dry heat on the immunity to transplanted cancer when exposure to heat was done 7 days and immediately before inoculation.

The foregoing experiments indicate a definitely higher resistance in the mice inoculated immediately after heating than in the controls, while the mice heated a week prior to inoculation show an even more pronounced degree of immunity. The blood count after the heat treatment showed a sharp but very transitory drop in the number of circulating lymphocytes, followed within 24 hours by a marked increase and a continuous rise in these cells lasting for a week or more. Histological examination of the lymphoid organs of heated

animals showed that by 48 hours after the treatment these organs contain a larger number of mitotic figures in the germinal centers than is normally seen. The height of the reaction, judging from the blood pictures and the condition of the spleen and lymph nodes, occurs about the 7th day after the exposure to the heat. The degree of immunity to the transplanted cancer seems to vary directly with the amount of stimulation of the lymphocytes existing at the time of or immediately following the inoculation.

SUMMARY.

It has been shown that resistance to transplanted cancer follows stimulation of the lymphoid tissue when the stimulation is induced by either heat or small doses of x-rays. In this paper we have attempted to determine whether the degree of immunity had a quantitative relation to the amount of the stimulation. Fortunately, the two methods at our disposal give stimulation of markedly different characters. The small dose of x-rays gives a sluggish lymphoid cell reaction of short duration with a definite latent period between the treatment and the evidence of marked stimulation, while after heat a short period of depression is followed by a sharp stimulation continuing over a much longer period. The cancer inoculation into groups of mice made immediately after exposure to x-rays shows little resistance, while the inoculation made at the height of the stimulation phase shows a definite increase in the immunity. Animals inoculated with cancer immediately after the heat treatment exhibit a pronounced immunity, but not so marked as that shown when the inoculation is made at the height of the stimulation.

The amount of resistance shown when the cancer inoculation is made at the height of the moderately stimulating effect following exposure to x-rays, is much less than that seen when the inoculation is made at the height of the heat effect when the degree of stimulation is much greater. When the lymphocytosis sets in after the tumor graft is established only a slight effect is noted. All these results together are taken to indicate that the degree of immunity is dependent on the amount of lymphoid stimulation existing either at the time of or following soon after the cancer inoculation.

STUDIES ON X-RAY EFFECTS.

VII. Effect of Small Doses of X-Rays of Low Penetration on the Resistance of Mice to Transplanted Cancer.

By WARO NAKAHARA, Ph.D., and JAMES B. MURPHY, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 22, 1920.)

Murphy and Morton[1] have shown that small doses of x-rays capable of stimulating somewhat the circulating lymphocytes increase the resistance to the growth of spontaneous tumors in mice. In their experiments the tumor was first removed by operation, the animal was treated with one small dose of x-rays, and the graft of the original tumor was returned to the left groin of the same animal, any direct effect of x-rays on the tumor being thus avoided. Studies of the stimulative effect of x-rays, generated by a Coolidge tube, on lymphoid cells showed that the dose of x-rays governed by the following factors will increase lymphoid activity in the rabbit:[2] $\frac{7}{8}$ inch spark-gap, milliamperage 25, distance from target 8 inches, and time of exposure 20 minutes. This dose with its original factors was not satisfactory when applied to another species of animal, namely mice, but by shortening the time of exposure it was possible to induce an active proliferation of lymphoid tissue in these animals.[3] The best results were obtained when the exposure was of 10 minutes duration.[4]

There have been accumulated numerous facts which point to the conclusion that the activity of lymphoid cells is a factor governing resistance to the growth of cancer. With the determination of the x-ray dose which suffices to induce lymphoid stimulation, another opportunity was offered to test the part played by lymphoid cells in this state of resistance.

[1] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[2] Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 75. Nakahara, W., *J. Exp. Med.*, 1919, xxix, 83.

[3] Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1920, xxxi, 13.

[4] Murphy, Jas. B., *Proc. Nat. Acad. Sc.*, 1920, vi, 35.

EXPERIMENTAL.

Young adult white mice were exposed to the following dose of x-rays: spark-gap $\frac{7}{8}$ inch, milliamperage 25, distance from target to back of mouse in the ordinary attitude 8 inches, and time of exposure 10 minutes. From 3 to 7 days afterwards the mice, together with a suitable number of controls, were inoculated subcutaneously in the left groin with a bit of a transplantable cancer (Bashford Adenocarcinoma No. 63). The rate of growth of the tumor was noted at weekly intervals thereafter.

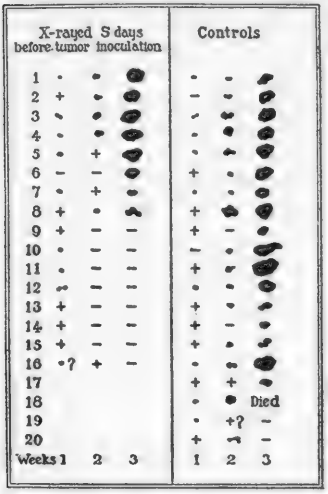
Table I gives the number of mice and the degree of resistance shown at the end of 3 weeks after inoculation (Text-figs. 1 to 3).

TABLE I.

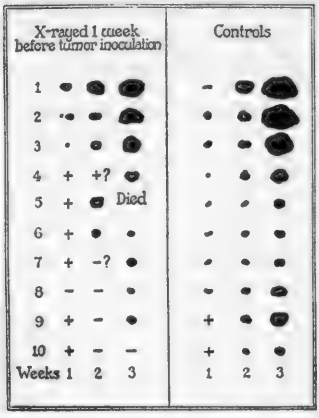
Experiment No.	Interval between exposure to x-rays and tumor inoculation.	Immunity in x-rayed animals.	Immunity in controls.
	days		
1	3	40.0 per cent (10 mice).	11.1 per cent (9 mice).
2	5	50.0 " " (16 ").	10.0 " " (20 ").
3	5	20.0 " " (10 ").	0.0 " " (10 ").
4	7	75.0 " " (8 ").	23.3 " " (9 ").
5	7	10.0 " " (10 ").	0.0 " " (10 ").
6	7	30.0 " " (10 ").	0.0 " " (10 ").
7	7	71.4 " " (21 ").	40.0 " " (10 ").

During the course of these experiments the virulence of the tumor used varied considerably. In Experiments 3, 5, and 6 the controls showed no resistance, and correspondingly the immunity was low among the x-rayed animals. Attention is called particularly to Experiment 5 (Text-fig. 2) in which, at the end of 3 weeks, only one animal in the x-rayed series showed absolute immunity. A definite retarding effect was evident, however, in that at the end of the 1st week all but two of the controls showed definite tumors, while only three of the x-rayed animals proved to be susceptible. By the 2nd week after inoculation all the control mice had palpable tumors and only five of the x-rayed animals. This experiment is a very good illustration of how the more virulent tumors may break through a

resistance. While the degree of resistance induced by this dose of x-rays is low, yet it is consistently higher in the treated animals than in the controls, even with the varying growth energy of the tumors.



TEXT-FIG. 1.



TEXT-FIG. 2.

TEXT-FIG. 1. Experiment 2. The rate of growth of Bashford Adenocarcinoma No. 63 in mice x-rayed before inoculation, contrasted with the rate of growth in untreated mice.

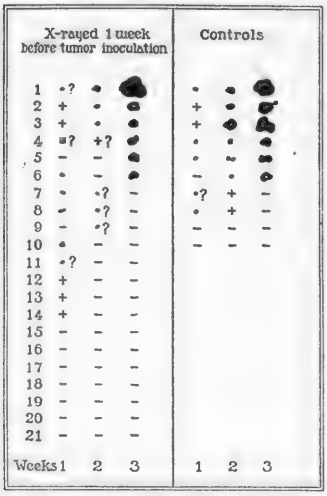
TEXT-FIG. 2. Experiment 5. The rate of growth of Bashford Adenocarcinoma No. 63 in mice x-rayed before inoculation, contrasted with the rate of growth in untreated mice.

DISCUSSION.

The foregoing experiments show conclusively that x-rays given in a dose sufficient to stimulate the lymphoid tissues, increase the resistance of mice to a transplanted cancer. As the degree of stimulation induced by this agent is less than that induced by exposure to intense dry heat or that following the injection of homologous living tissue, it is not surprising that the degree of resistance is less than that seen after the employment of the latter two methods.

It is of interest to note that the degree of resistance exhibited by the x-rayed mice varied with the growth energy of the tumor as shown by the number of takes in the control animals.

Our original observation that x-rays in small amounts increased the resistance of a mouse to transplants of tumors,[1] was later confirmed by Russ, Chambers, Scott, and Mottram,[5] who used transplants of the Jensen rat sarcoma with rats as their subjects. They gave an exposure of 12 seconds repeatedly, instead of the single dose which we have used.



TEXT-FIG. 3. Experiment 7. The rate of growth of Bashford Adenocarcinoma No. 63 in mice x-rayed before inoculation, contrasted with the rate of growth in untreated mice.

SUMMARY.

A relatively increased degree of resistance in mice to a certain strain of transplantable cancer was demonstrated after treatment of animals with small doses of x-rays capable of stimulating lymphoid tissue. The refractory state induced was determinable 3 to 7 days after the dose of x-rays was given.

[5] Russ, S., Chambers, H., Scott, G. M., and Mottram, J. C., *Lancet*, 1919. i, 692.

STUDIES ON X-RAY EFFECTS.

VIII. Influence of Cancer Inoculation on the Lymphoid Stimulation Induced by Small Doses of X-Rays.

By WARO NAKAHARA, Ph.D., and JAMES B. MURPHY, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, December 31, 1920.)

The studies carried out in this laboratory have shown that immunity to cancer, whether natural or induced, is attended by lymphoid stimulation.[1] X-rays have been employed to secure the stimulative effect on the lymphoid elements;[2] when of sufficient amount, they have been found to increase[3] the resistance of mice to cancer transplants[4] made at the height of the reaction. On the other hand, if the cancer inoculation is made immediately after the x-ray treatment, no unusual degree of resistance is exhibited.[5] Hence it would appear that the early inoculation of cancer must have in some way interfered with the development of the defensive mechanism. The present study has been planned to elucidate this point.

EXPERIMENTAL.

Experiment 1.—Twelve normal white mice were given the following dose of x-rays (Coolidge tube): spark-gap $\frac{7}{8}$ inch, milliamperage 25, distance 8 inches, and time of exposure 10 minutes. The manner of applying the dose was the same as in experiments previously reported. As soon after the treatment as possible the animals were inoculated subcutaneously in the left groin with fragments of a Bashford adenocarcinoma, No. 63. Of these mice, six were killed for histo-

[1] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[3] Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1920, xxxi, 13.

[4] Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1921, xxxiii, 429.

[5] Murphy, Jas. B., *Proc. Nat. Acad. Sc.*, 1920, vi, 35. Murphy, Jas. B., Nakahara, W., and Sturm, E., *J. Exp. Med.*, 1921, xxxiii, 423.

logical study 48 hours and the other six 4 days after the inoculation. No difference was noted at autopsy between the two groups of mice killed at different periods.

In three of the six animals in each group the spleen and lymph nodes were found at autopsy to be smaller than is usual in normal mice. The mesenteric node, which is the largest lymph node in the mouse, was in a few instances as small as the normal inguinal, or even the axillary node.

Microscopically, a considerable number of pycnotic cells were found in these organs, but the deposit of pigment in the spleen was never conspicuous. The most striking feature was the almost complete suppression of the proliferative activity of lymphoid cells in half of the animals examined, only a few mitotic figures being found in each section. This suppressed activity was equally evident in the two groups, and apparently was independent of the size of lymphoid organs. In the remaining half of the animals, mitotic figures were found more frequently, but in no instance was there any sign of an activity above normal.

Experiment 2.—Eleven normal white mice were given the same dose of x-rays as before; six were killed 48 hours and five 4 days after the treatment, without having been inoculated with the cancer.[6]

The increase in the number of mitotic figures in the lymphoid tissue was evident in the majority of cases; namely, in nine out of eleven animals. The remaining two animals showed only slight signs of lymphoid proliferation, judging by the limited number of mitotic figures found.

Experiment 3.—Eleven normal white mice were treated with the same dose of x-rays. 7 days later the animals were inoculated subcutaneously in the left groin with fragments of Bashford Adenocarcinoma No. 63. Six of these mice were killed after 48 hours and five after 4 days; *i.e.*, 9 and 11 days after the x-ray treatment.

No unusual macroscopic feature of the lymphoid organs was noted at autopsy. There was some variation in the histologic condition of these organs. In six of the eleven animals, there was an extensive stimulation of lymphoid tissue as evidenced by the large number of mitotic figures in the tissue. The mitotic figures were found abundantly, not only in the area of the germ centers, but also in the pulp. The nodules were more or less enlarged. There were few pycnotic cells, and pigment was almost entirely absent. In four other animals, the stimulative reaction was similar in kind but somewhat less pronounced. One animal was exceptional in that it showed almost no signs of cell proliferation.

Experiment 4.—Six normal white mice were treated with the same dose of x-rays as those above but were not inoculated with cancer. Three were killed 9 days and three 11 days afterwards.

The spleen of four out of the six mice was found at autopsy to be abnormally dark in color and, except in one animal in which it was below normal, of usual size.

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[6] This was a repetition, for the sake of comparison, of an experiment previously reported.[3]

Histologically, the dark spleens showed a great accumulation of blood in the pulp, which was sparingly supplied with lymphoid cells. In contrast to the findings in Experiment 3, mitotic figures were in no instance abundant in the lymphoid tissue of spleen or of the lymph nodes. Cells with pycnotic nuclei were numerous, except in one specimen in which the number was small.

For a further comparison, the four preceding experiments were repeated in a single experiment which included groups subjected to the various experimental conditions of the individual experiments described above.

Experiment 5.—Twenty-five normal white mice, divided into four groups, were given the same dose of x-rays as in the previous experiments.

Group 1: eight mice were inoculated with Bashford Adenocarcinoma No. 63 immediately after the treatment with x-rays. They were killed 3 days later.

Group 2: seven mice were killed 3 days after the x-ray treatment without having been inoculated with cancer.

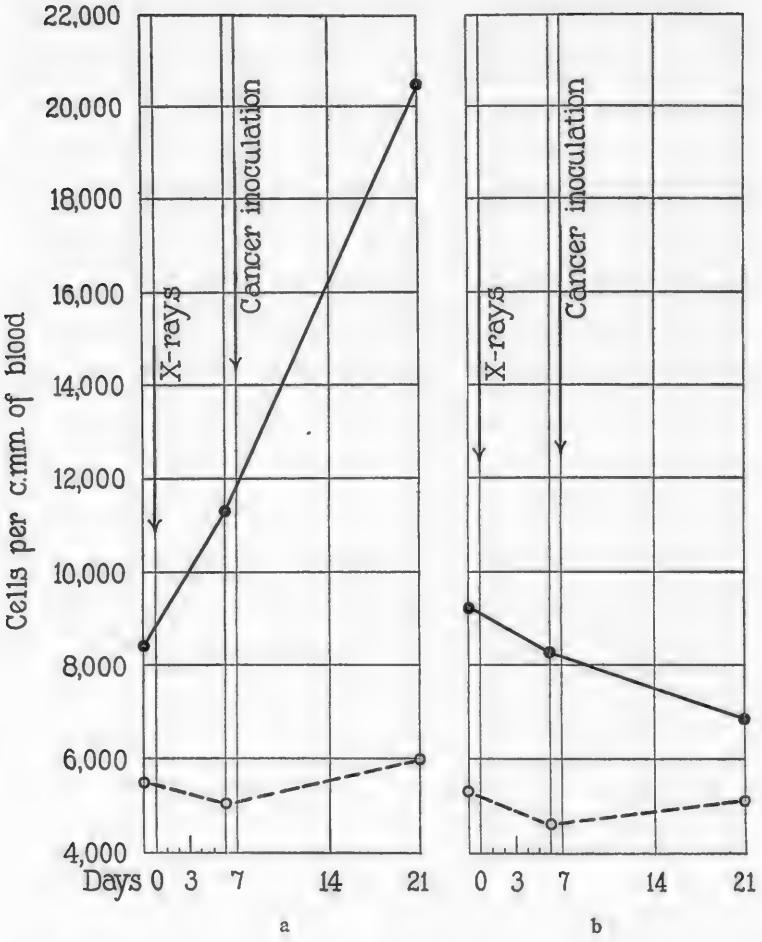
Group 3: six mice were inoculated, 7 days after the treatment with x-rays, with the same strain of cancer and were killed 3 days after the inoculation; *i.e.*, 10 days after the x-ray treatment.

Group 4: four mice uninoculated with cancer were killed 10 days after the x-ray treatment.

Of Group 1, all the mice except one individual showed suppression of the usual proliferation of the lymphoid elements. Of Group 2, all showed definite signs of increased proliferation of the same elements. In Group 3, three mice showed evidences of extensive stimulation, while the remaining three had a less marked reaction. Group 4 showed little signs of lymphoid proliferation.

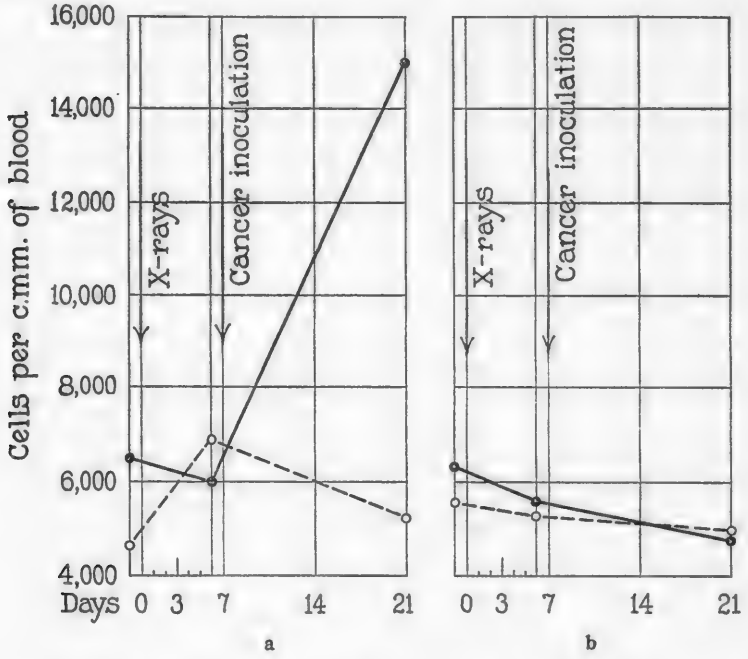
Hence, the results of this experiment are in agreement with those of the four experiments immediately preceding.

The following conclusions can be deducted from the results. (1) Cancer inoculation made immediately after a stimulative dose of x-rays interferes with the lymphoid reaction and little or no stimulation results. (2) Cancer inoculation made at the height of the stimulation augments the lymphoid reaction, and the proliferative activity of the cells continues longer than in animals which have been stimulated but have not received the cancer inoculation. (3) A proportion of animals given the stimulative dose of x-rays fails to react. It is of considerable interest to ascertain whether mice which fail to react are susceptible to cancer inoculations. To determine this point we have used the blood counts to ascertain the absence or presence of a stimulative phase.



TEXT-FIG. 1, *a* and *b*. Experiment 6. Composite curves of white blood cell counts on mice x-rayed and inoculated with cancer 7 days later. (*a*) Composite curves from fourteen mice proved to be immune. (*b*) Composite curves from ten mice proved to be susceptible. ———— Lymphocytes. ---------- Polymorphonuclear leucocytes.

Experiment 6.—Blood counts were made on twenty-four mice which were then given an exposure of x-rays similar to that used in the preceding experiments. 7 days later a second count was made, followed by the inoculation of each animal with a graft of the Bashford mouse cancer, and 14 days later a third count. Fourteen of the twenty-four animals resisted and ten responded to the cancer inoculation.



TEXT-FIG. 2, *a* and *b*. Experiment 7. Composite white blood cell counts on mice x-rayed and inoculated with cancer 7 days later. (*a*) Composite curves from the immune mice. (*b*) Composite curves from the susceptible mice. ———— Lymphocytes. ---------- Polymorphonuclear leucocytes.

In the immune mice (Text-fig. 1, *a*) the average number of lymphocytes per c. mm. of blood before x-ray treatment was about 8,300, the number of polymorphonuclear leucocytes being about 5,400. 6 days after the treatment (1 day before cancer inoculation) lymphocytes and polymorphonuclear cells were about 11,400 and 5,000 respectively. 2 weeks after the cancer inoculation the lymphocytes had, however, risen to approximately 20,000, while the polymorphonuclear cells showed but little change, being about 6,000.

In susceptible mice (Text-fig. 1, *b*) the average number of lymphocytes per c. mm. of blood before the dose of x-rays was approximately 9,600, the average number of polymorphonuclear cells was 5,300. A slight decrease in the white cells was noted 6 days after the treatment, the lymphocyte and polymorphonuclear leucocyte counts being 8,000 and 4,600 respectively. 2 weeks after the cancer inoculation the lymphocyte count was 7,000, the polymorphonuclear cell count 5,000.

Experiment 7.—The preceding experiment was repeated with twenty-six x-rayed mice, only nine of which resisted the cancer inoculation. Blood counts were made in the same way as before.

The average number of lymphocytes per c. mm. of blood in immune mice (Text-fig. 2, *a*) before x-ray treatment was about 6,500, and of polymorphonuclear leucocytes about 4,500. 6 days after the x-ray treatment the lymphocytes showed no material change, but the polymorphonuclear leucocytes went up to about 6,500. 2 weeks after the inoculation of cancer there was a rise of lymphocytes to about 15,000, while the polymorphonuclears decreased to about 5,000.

In susceptible mice (Text-fig. 2, *b*) the average number of lymphocytes and polymorphonuclear cells per c. mm. of blood before exposure to x-rays was about 6,000 and 5,000 respectively. No material change in these numbers was observed 6 days after x-rays. 2 weeks after cancer inoculation, however, the lymphocytes and polymorphonuclear cells were slightly decreased in number, the former being about 4,000 and the latter about 4,500.

DISCUSSION.

A comparison of the experiments described leads to the conclusion that if cancer inoculation is made immediately after the stimulative treatment with x-rays, no lymphoid stimulation occurs such as would regularly occur if the cancer inoculation were not made. On the other hand, if cancer inoculation is made 7 days after the x-rays are given, thus allowing the stimulation to develop before the inoculation, there is in the majority of cases what might be called a second stimulation of lymphoid tissue. It is significant, in connection with these facts, that while only little resistance to the transplantation was discovered when cancer was inoculated immediately after the x-rays, evidence of increased resistance appeared when inoculation was postponed until the 7th day.[5]

Attention is drawn in this connection to the parallelism existing between the lymphoid reaction accompanying the immunity to cancer grafts induced by physical agents (x-rays) and that induced by a biological agent (homologous blood). We have already shown

that mice immunized to cancer by means of an injection of defibrinated blood show an increase in the number of mitotic figures in the lymphoid tissue. Such mice, when inoculated with a cancer graft 10 days after the injection exhibit a second stimulation of the tissue,[7] as well as a marked blood lymphocytosis.[1] The experiments reported indicate a corresponding effect brought about by small doses of x-rays. The blood counts on the animals after the cancer inoculation show that only the animals presenting an increase in the lymphocytes prove to be resistant to the cancer. However, it should be stated that the blood counts were not made at a time to show the primary stimulative effect of x-rays, since this reaction, as previously shown, is of short duration. Hence it appears that as a result of the primary stimulation the animals have acquired the ability to react more strongly to a second stimulation; namely, the cancer inoculation.

SUMMARY.

Mice treated with small doses of x-rays and inoculated with cancer immediately afterwards, show a marked suppression of lymphoid proliferation. If, however, the cancer inoculation is made 7 days after the exposure to x-rays, thus permitting the primary lymphoid stimulation known to occur soon after the x-ray treatment to arise, a second stimulation takes place in a large proportion of mice thus inoculated.

Changes in the blood of mice x-rayed and inoculated with cancer 7 days afterwards show that the state of resistance to cancer inoculation is attended by blood lymphocytosis, as is the case in all other varieties of immunity to transplanted cancer so far studied.

[7] Murphy, Jas. B., and Nakahara, W., *J. Exp. Med.*, 1920, xxxi, 1.

THE ETIOLOGICAL RELATION OF BACILLUS ACTINOIDES TO BRONCHOPNEUMONIA IN CALVES.

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PLATES 42 TO 50.

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In the early months of 1917 an outbreak of pneumonia occurred among calves belonging to a large dairy herd in which about 100 calves are raised annually. Up to May 1, eleven cases had been autopsied. Nine of these were killed in advanced stages of the disease and two died. Scattering cases occurred at the rate of about one a month into November. Of these, one died and seven were killed. In the course of the investigations a minute bacillus was isolated whose etiological relation to the pneumonia was left in doubt.[1] In this outbreak a peculiar disease of the kidneys occurred in ten out of the nineteen cases. The lesions were situated in the cortex and consisted of foci of sclerosis with destruction of the secreting tissue.

More than 2 years later, in October, 1919, there began in the young stock of the same herd a similar series of cases. Up to March of 1920, ten cases came to autopsy. Of these four had died and six were killed. The kidney lesions were absent. A belated case was observed in June. There may have been more cases which recovered without being detected, since no careful clinical examination was made and the disease was recognized only when the respiration had become sufficiently abnormal to warrant removal from the herd. The ratio of animals openly diseased to the entire number of calves was relatively low. This may have been due to the fact that the calves were kept in groups of six to eight in adjoining open pens, those of nearly the same age being kept together.

[1] Smith, T., *J. Exp. Med.*, 1918, xxviii, 333

In the second outbreak the lesions of the respiratory tract, more particularly of the lungs, were the chief if not the only ones. In the first, as stated above, a nephritis was associated with over half the cases. In the present article references to the nephritis will be omitted and made the subject of a separate paper. A description of the bacillus was published in 1918.[1] Its chief distinguishing characters are briefly as follows:

1. Multiplication in the form of small flakes up to 1 mm. in diameter which consist of parallel bundles of filaments, each filament terminating in two club-like expansions, one at each end. The flake thereby becomes more or less rounded, mulberry-like, and resembles a sphere with clubs projecting from the central mass. In this *Actinomyces*-like growth and within the sheathing filaments are chains of minute bacilli.
2. The sheaths and clubs are produced in the condensation water of coagulated serum and to a less extent on the sloped surface but not on agar plus tissue or blood. On these latter media the growth is feeble and the organism appears in the form of rods without the sheaths. It often fails entirely.
3. On ordinary media such as plain agar and bouillon the bacillus multiplies very faintly or not at all.
4. After 10 or more days growth on the agar plus blood or tissue, minute refringent bodies are found free in large numbers and also within the remaining rods. In some cultures all bacilli had disappeared. This phenomenon has been tentatively interpreted as spore formation.
5. The bacillus fails to produce any appreciable lesions in small animals.

Pathological Anatomy and Bacteriology of Individual Cases.

The gross appearances of the lungs differed very much from animal to animal but the cases could be grouped according to certain characters into acute, chronic, and intermediate types. The important features of the pneumonia, both pathological and bacteriological, are best presented by a brief description of certain cases which will form the basis for a discussion of the etiology. For the names applied to the different lobes of the cow's lungs in these pages, the reader is referred to the description of Text-figs. 1 and 2, page 450.

No. 440.—Female calf, aged 49 days. Died suddenly Nov. 12, 1919, and was autopsied within a few hours. There is no history of any pronounced illness preceding death, although this may have been overlooked by the attendant.

The lesions are restricted to the respiratory organs. The lungs are affected symmetrically. Both cephalic and ventral lobes are solidified with the exception

of six or more small air-containing regions along caudal border of right cephalic lobe and several along dorsal margin of the same lobe. Near root of right ventral lobe there is an air-containing region several centimeters in diameter. The large caudal lobes are solidified in the cephalic third or half. The involved tissue covers much more of the diaphragmatic than of the dorsal surface of these lobes. Air-containing lobules encroach on the hepatized territories and foci of collapsed lobules extend into and permeate the air-containing portions. The small azygous lobe consists of inflated and dark red collapsed regions intermingled.

The involved lobes are as firm as liver tissue to the touch. Their dimensions are about twice those of lungs normally collapsed. The gross appearance of the pneumonic regions differs from lobe to lobe. The cephalic and ventral lobes show closely set, grayish dots 1 to 3 mm. in diameter on a dark red ground, excepting a strip comprising the dorsal border, where the tissue is uniformly dark red, lacking the lighter dots. Similarly, the caudal lobes show the mottled character in a strip adjacent to the ventrals and the smooth, more uniform condition caudally, bordering on the still air-containing regions of these lobes. The mottling is also found on section. Embedded in one ventral lobe there are several foci, 4 to 5 mm. in diameter, which are made up of a semisolid caseous matter. Quite small foci 1 to 2 mm. in diameter of similar character are found here and there in the other lobes.

Larynx and trachea are normal. At bifurcation there is a mass of whitish mucoid substance. Both bronchi are deeply congested and flecks of whitish, soft, mucoid material are scattered over the mucosa. When the lobes are cut across and gently compressed, minute whitish molds are forced out of the smaller bronchioles. The lymph nodes, both mediastinal and bronchial, are somewhat enlarged, on section slightly congested and juicy. Occasional whitish, point-like foci are seen on the cut surface. The pleura is normal. The heart is flabby; pericardium normal. In the fat around the base of the right ventricle there is some patchy hemorrhage. Both sides of the heart contain large, dark, soft clots extending as cylindrical molds into all the large vessels.

Sections from fixed and hardened tissue of the various lobes stained in eosin and methylene blue present the following features. The small air tubes are filled with a mixture of cells and a peculiar, faintly bluish red, homogeneous material, probably mucus. The relative amount of this and of cellular material varies from tube to tube. Within the parenchyma there are foci consisting each of a group of alveoli which are packed with cell masses and the homogeneous substance referred to above. Among the desquamated alveolar epithelial cells are smaller cells the nuclei of which have contracted into irregular star-shaped, solidly stained masses of chromatin or into other irregular shapes, simulating the nuclei of polynuclear leucocytes. They are either lymphocytes or endothelial cells. A few polynuclear leucocytes permeate the mass of cells and debris. The cell foci may be so numerous as to coalesce, or there may be between them a zone of alveoli in which only a few desquamated alveolar cells are seen. It is these cell foci which appear to the naked eye as the grayish subpleural dots. The cell masses in the bronchioles and the alveoli are the same in character.

Another feature of significance is the presence within the smallest air tubes of what appear to be ingrowths from the wall (Figs. 1 to 3). These ingrowths consist of cells of the types described as present in the alveoli, embedded in a feebly stained matrix. The epithelium is missing where the ingrowth takes place. Within this matrix are dense masses of bodies varying in shape from minute rods in small colonies to groups of roundish bodies 2 microns in diameter with only the outline stained (Figs. 4 to 7). These latter bodies are also found in the alveolar cell masses but much less abundantly than colonies of minute bacilli (Figs. 8 and 9).

The origin of the cloud-like masses of bacteria pushing into the lumen of the air tube is obscure. A prolonged examination of sections from this and other cases makes it probable that the bacteria start in the cytoplasm of the epithelial cells, develop into colonies, and thereby cause a great increase in the size of the cells and their eventual destruction. The mass thus projects into the lumen where some of the migrating cells fuse with it, producing the characteristic obstructing plug. It is doubtful whether there is any actual hyperplasia of the epithelial cells. In the cell foci of the parenchyma a similar invasion of the alveolar cells takes place, leading to destruction of such cells and the appearance of hemorrhages and of the lymphocyte cell masses.

Cultures were made on slanted coagulated horse serum and slanted agar with and without a few drops of horse blood. All tubes were hermetically sealed and incubated. The cultures were made both by transferring bits of lung tissue and by inoculating the tubes with a heavy platinum wire thrust into the pneumonic tissue. After several days, growth appeared and in all but a few tubes *B. actinoides* was present in pure culture. In the remaining tubes a few foreign colonies were also present.

No. 450.—Black and white female calf. Born Oct. 27, 1919. Attendant reported that on Nov. 27 the calf did not eat its food and that breathing was rapid. From that day on the temperature was taken several times and found to be around 40.5°C. There was much coughing, the respirations continued short and superficial, and emaciation progressive. Dec. 4. The calf, then weighing 85 pounds, was killed by stunning with a heavy blow, clamping the trachea to prevent aspiration of stomach contents into the lungs, and opening the vessels of the neck. There was nothing noteworthy about the organs excepting the respiratory tract.

When the thorax was opened the lungs collapsed with force. The distribution of the pneumonic lesions is somewhat less extensive than in the preceding case. Both cephalics and ventrals are entirely consolidated. The cephalic half of the azygous lobe and a narrow strip along the cephalic margin of the right caudal lobe are involved. The left caudal lobe is intact. There is a narrow margin of air-containing lung tissue along the dorsal ridge of both lungs almost to the cephalic tip, also small inflated lobules in the left ventrocephalic lobe.

The condition of the consolidated lobes is not the same throughout. The right cephalic and the right ventral lobes are much larger than the normal collapsed

condition would be. They are grayish red to grayish yellow in appearance. The remainder of the affected lung is but a trifle larger than in the normal collapsed state and dark red with a faint, regular mottling of a lighter color. In this lung the grayish dots or granulations characteristic of the preceding case are absent. The pleura is normal.

The lower portion of the trachea and the main bronchi at the bifurcation contain masses of a viscid, opaque, whitish, dough-like mucopus. The same glairy, pearly white masses can be expressed from the cut ends of the minute air tubes of the dark red lobes. Only a small amount is expressible from the air tubes of the large grayish lobes. This glairy matter is very tenacious and breaks up into fragments when crushed between cover-glasses. The bulk of the material is made up of polynuclear leucocytes. The dark reddish tissue is quite moist when rubbed on covers, the grayish less so. The latter leaves on the cover-slip a smeary, milky film. Projecting from the right ventral lobe is a whitish mass the size of a pea. It is made up of a thin walled cyst filled with a readily dislodged, dough-like mass. Cover-glass films show abundant alveolar epithelium, polynuclear leucocytes, and several dense masses of very fine, rather feebly stained bacilli. Films from tracheal and bronchial mucopus and from lung parenchyma contain the same cellular and bacterial elements.

The difference between the gross pictures of the lungs of Nos. 440 and 450 is paralleled by differences in the microscopic picture. The peculiar cell infiltration in No. 440 has disappeared and polynuclear leucocytes have taken its place. The cell plugs in the air tubes contain chiefly polynuclears among which are scattering alveolar cells. The peculiar amorphous, homogeneous material which was regularly associated with colonies of bacteria in No. 440 is absent and bacteria are not detected. The parenchyma is the seat of polynuclear infiltration in foci which coalesce in the older stages and in the more recent stages gather irregularly in the collapsed tissue. The invasion of new territories is characterized by a ring of fibrin-blocked alveoli around the invaded air tube and occasionally hemorrhages. The interlobular tissue contains oval masses of fibrin lodged in the distended lymph spaces.

The difference between the two cases is further indicated by a new feature. All small air tubes are surrounded by a loose zone of cells which resemble plasma cells in form and the basophilic character of the cytoplasm and which collect in the subepithelial tissue.

Cultures prepared from the different lobes by transferring bits of lung tissue to horse serum, agar and blood, and plain agar, as well as by inoculating the same kind of media with heavy and fine platinum wire forced into the lung tissue yielded cultures of *B. actinoides* which were pure with the exception of those from the azygous lobe in which a few large streptothrix-like colonies appeared. The tubes inoculated with the heavy platinum wire developed, whereas most of those inoculated with a fine wire remained sterile.

No. 446.—This is a case which duplicates in all particulars No. 450 (Fig. 14). This calf was born in September and was 2 months and 2 days old when killed.

Cultures prepared from the different lobes in the manner described for No. 440 developed, with few exceptions, growths of *B. actinoides*. A few stray colonies of other kinds were present in several tubes.

No. 432.—This case may be classed as intermediate between the first and the following group. Female, born June 21; killed Oct. 21, when 4 months old. The meager data indicate that it was sick with some respiratory affection late in July and early in August. Early in October the respiration became short and quick, there was frequent coughing, and the calf breathed with mouth open. The respirations continued short and labored until it was killed. The temperature fluctuated between 39° and 40°C. The animal was killed by a stunning blow on the head, clamping trachea, and severing vessels of neck.

The consolidation of the lungs is of the usual extent. The pneumonic process involving completely the cephalic half of both lungs has invaded both caudal lobes and is separated from the air-containing tissue by a sharp, irregular, jagged line. Pneumonic lobules also appear as islands in these lobes. The trachea contains whitish, mucoid masses and the small air tubes of the affected lobes are filled with molds of similar material.

The hepatization is very firm, the affected lobes about twice the dimensions of the normal collapsed state. The surface is mottled like that in No. 440. Necrosis is absent. The cut surface shows the walls of the small air tubes distinctly thickened. In general the gross appearance indicates that the process is more or less of the same age throughout, except in the caudal lobes, where it is freshest. The mucopurulent molds from the small air tubes and the loose masses in the trachea contain large numbers of very minute and somewhat larger bacilli. Histological examination of sections from the different lobes indicates a transition in the pathological process from that of No. 440 to that of No. 450. In some regions the infiltrating cell masses are mononuclear, the contents of the minute air tubes largely made up of the homogeneous, bluish stained substance and groups of minute bacilli. In others, polynuclears predominate both in the parenchyma and the air tubes, and bacteria are rare or absent.

The bacteriology of the processes in this lung is complicated. On the agar and blood agar slants many roundish, rather fleshy colonies develop which have the cultural characters of the hemorrhagic septicemia group but with very low virulence. The general presence of *B. pyogenes* is indicated by liquefying colonies in all horse serum tubes. A third type of bacilli not identified was present in some tubes. Identifiable by its peculiar growth, *B. actinoides* was demonstrated as occurring in most tubes, but repeated attempts to obtain it in pure culture failed, owing to the predominance of the other types.

A type of pneumonia more acute than that represented by No. 440 was observed in three cases. No. 429, the first of the present outbreak to come to autopsy, was born September 7 and died October 12. The second, No. 436, was born September 20 and died

November 4. The third, No. 447, was born October 17 and died November 29. The three calves were thus respectively 35, 45, and 43 days old at the time of death. The three cases present certain differences among themselves.

No. 429.—All lobes are much enlarged over the normal collapsed size. There is an adhesion of most of cephalic and ventral lobes to ribs and to pericardium. The attachments are easily broken except the adhesion of right cephalic lobe to pericardium which is not separable. Consolidation of all but one-half of right caudal and two-thirds of left caudal lobe. There is a general faint putrefactive odor emanating from the lungs. The affected lung tissue is dark red and sprinkled over with numerous yellow, cheesy foci 2 to 5 mm. in diameter and projecting slightly above the pleura. These foci permeate the lung tissue with the exception of the caudal and azygous lobes in which such foci are few and small. Besides the small foci the right cephalic lobe contains a sequestrum 2 by 2 by 5 cm., yellowish, and firm like rubber. The trachea is uniformly reddened and covered with whitish, viscid flakes.

No. 436.—Extent of lung involvement and size of affected lobes are as in No. 429. The lung tissue resembles that of No. 440. It is sprinkled over densely with lighter grayish foci about 0.5 mm. in diameter. On pressure very little fluid and only a few consistent molds can be expressed from the air tubes. The multiple necroses found in No. 429 are absent. The mucosa of the trachea is uniformly congested, the bronchi deeply so, bordering on hemorrhage. The mucosa has on it some very thin patches of a pseudomembrane.

No. 447.—The involvement is somewhat less extensive than in Nos. 429 and 436. The pneumonic lobes are firm, only moderately larger than the normal collapsed state, uniformly dark red, and interspersed with a few inflated lobules. There is no distinct mottling with lighter dots as in No. 436, but the surface is rather variegated with larger patches of lighter color. Necrotic foci are not found. The trachea is clean and normal to the bifurcation where some flour-paste-like masses of mucopus are lodged. The main bronchi of affected lobes are filled with a similar thick pasty mass but those of the caudal lobes are free.

The microscopic picture in these three cases as constructed from sections of the different lobes is very much like that of No. 440 already described. The consolidation is due to focal infiltration of the parenchyma with the type of cell described under No. 440. Polynuclears are scarce or absent. The minute air tubes contain, besides cell masses, the homogeneous, bluish stained material, and it is in and among this material that masses of minute bacteria occur. The necroses of No. 429 occupy variable areas of lung tissue in which the alveoli have become impacted with the cell type mentioned. In these cases the gathering of plasma cells in the mucous membrane of the air tubes had not yet begun.

Cultures made from different parts of the lungs of the three cases indicated the presence of various species. Present in all three was *B. pyogenes*, especially abun-

dant in No. 429, less so in No. 436, and least so in No. 447. The sections containing the necrotic foci of No. 429 stained according to Gram-Weigert showed large, deep blue spots resolved as minute bacilli and evidently colonies of *B. pyogenes*. Several other types of colonies were found in the cultures. *B. actinoides* could not be detected in cultures from Nos. 429 and 436. Several cultures from No. 447 contained large numbers of colonies of *B. actinoides* but pure cultures failed because of the presence of *B. pyogenes*. Inasmuch as the type of disease was the same in the three cases, it is probable that *B. pyogenes*, being an acid producer, interfered with the development of *B. actinoides*.

Another group of cases is represented by calves in which the disease processes have gone on more slowly and to a much farther stage. The involved tissues have become necrotic and encapsuled, causing firm adhesions of the pleura to the chest wall and pericardium. There is a broad zone of plasma-like cells around the smaller air tubes, varying in size with the age of the calf.

Case 455.—Female calf born July 26; 4 months old lacking 3 days when killed. The attendant reported that this calf was sick with scours in August. Late in September it had a cold and in November symptoms of pneumonia were present. When killed it was very thin, the respirations short and labored. The cough was frequent, the temperature 40°C.

In this case also the pathological changes were limited to the respiratory organs. When the sternum was removed, the lungs collapsed vigorously. The pneumonic process had the usual extent and symmetrical distribution. The appearance of the lobes was much the same. The tissue is a reddish yellow and beset with closely crowded grayish areas, about 2 mm. in diameter, although varying more or less in size. Similar areas are seen on the cut surface. In the right cephalic lobe the grayish areas are larger and tend to coalesce. In the free tip of this lobe there is a group of larger foci about pea size, made up of thin walled sacs containing what appears to be in part necrotic lung tissue, in part mucus and pus. The pleura covering these foci gives rise to delicate fibers attaching lobe to pericardium. The mucosa of the trachea is normal and has lying in it white, curdy, viscid masses 4 to 5 mm. in diameter made up of polynuclear cells, alveolar epithelium, mucus, and some minute bacilli. All the small air tubes in the consolidated lobes when compressed exude thick, glairy, pearly white masses. Microscopically this material is like that in the lower trachea. All thoracic lymph nodes are distinctly larger than normal; on section quite moist and exuding a milky fluid.

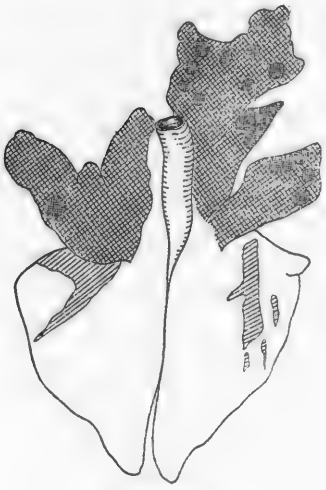
The histological picture differs in degree from that of the more acute types. The most striking character is the presence of a zone of plasma cells under the epithelium of the air tubes, varying in thickness and especially broad in some lobes. The smallest air tubes are distorted, or else nearly obliterated so that their presence is only indicated by the plasma cell groups and zones. These cell zones plus

a broadening of the submucous layer probably represent the grayish areas seen from the surface and on section. The lung tissue around and between these zones is either slightly inflated and contains some desquamated cells, or else is collapsed or filled with polynuclear leucocytes. In sections bacteria are not seen, except in one large mass growing into and nearly obliterating the already dilated and deformed tube. In this mass colonies of fine, rather feebly stained bacilli are present. The flora of the pneumonic lobes of this case resembles somewhat that of No. 432. Of the large number of cultures, about thirty in all prepared with minute and pea size bits of lung tissue, all showed development. The colonies in each tube were few in number. *B. pyogenes* predominated in being present in most tubes. A second form, consisting of 2 to 3 mm., smooth, grayish, translucent colonies which tended to flow down the slant, was probably a variety of *B. bovisepticus*. Several other forms were present. *B. actinoides* was detected in only two cultures among other forms. Among several cultures from bits of tissue from thoracic lymph nodes, one contained *B. pyogenes*.

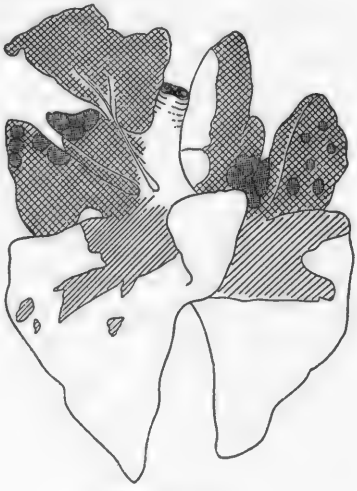
Case 462 (*Text-Figs. 1 and 2 and Fig. 12*).—Female, born Nov. 4; killed Jan. 7, weighing at this time 102 pounds. It began life with scours which persisted for some weeks. Evidences of respiratory trouble began to appear Dec. 27. Although only a few days over 2 months old when killed, it presented advanced destructive lesions in larger number than any other case of this small outbreak. The pathological changes, if we except the general disappearance of fat deposits, were restricted to the lungs. Both cephalic and ventral lobes and a small adjacent zone of both caudal lobes were involved. The smaller lobes were adherent to the chest wall and pericardium through thin bands severed with some difficulty. In all but the caudal lobes are isolated or agglomerated, whitish, projecting nodes 5 to 10 mm. in diameter. The lung tissue between them is dark red and beset with paler grayish areas 2 to 3 mm. in diameter. The nodes are sacs containing a glistening, pearly white, viscid, flour-paste-like mass, rather sticky and not easily spread on cover-slips. It consists of cellular elements among which are dense masses of minute bacilli. Within this mucoid, whitish coating is a nucleus consisting of a lobulated mass not so white as the mucoid covering, more brittle, and finely spongy. The gross and microscopic characters indicate necrotic lung tissue. The walls of the cavities containing the sequestra are 1 to 1.5 mm. thick, and smooth. Where a number of cavities are close together they evidently communicate, since pressure continues to force the necrotic and mucoid masses out of all the cavities through one opening. Stained films of tracheal mucopus and pus from the necrotic foci show besides cellular elements many minute bacilli frequently in dense masses. The air tubes of the affected lobes contain the same viscid, mucoid, purulent masses found in preceding cases.

The histological changes found in the various lobes do not differ materially from those of the advanced, chronic type. In general there is much collapse of the parenchyma with marked broadening of the alveolar walls due to infiltration of endothelial cell types. The collapsed tissue is focally occupied by dense infiltrations of polynuclear cells into the alveoli. In some lobes the normally distended

alveoli contain sparse collections of desquamated cells and polynuclears. The air tubes in some lobules have a dense enveloping zone of plasma cells. In others it is very slight. Sections through the necrotic foci show sequestra of lung tissue enclosed in a dense zone of plasma cells and more or less connective tissue stroma. Bacteria colonies are not seen.



TEXT-FIG. 1.



TEXT-FIG. 2.

Diagrams of involved lung tissue, Calf 462.

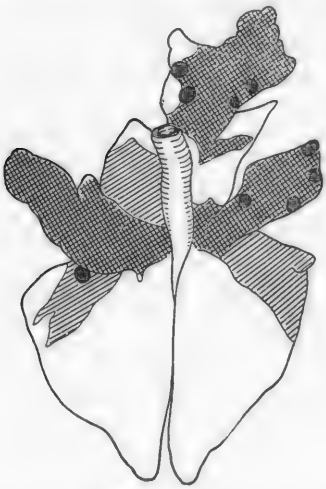
TEXT-FIG. 1. Dorsal aspect.
TEXT-FIG. 2. Ventral or diaphragmatic aspect.

In the text the lobes are designated cephalic, ventral, and caudal. In the right lung the cephalic lobe is relatively very large and has a separate bronchus. In Text-fig. 2 the small median or azygous lobe is shown. The normal lung tissue is unshaded; the heavily shaded circles represent necrotic tissue; the areas cross-hatched designate an older stage than the areas having parallel lines only.

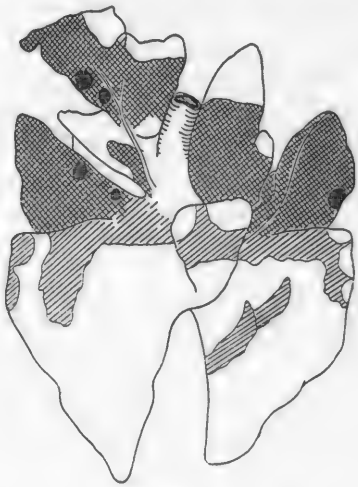
Cultures were prepared from the different lobes on several media as described under Case 440. The extensive necrosis of lung tissue naturally led to the inference that a variety of bacteria would be found. This, however, was not the case. In none of nine horse serum tubes did any liquefaction suggesting *B. pyogenes* occur. In all but one, which remained sterile, *B. actinoides* multiplied in pure culture. Similarly the plain agar tubes containing bits of tissue and blood agar tubes developed pure cultures of *B. actinoides* with two exceptions in which a mold and a fleshy colony appeared.

The following case was the last of this particular outbreak.

No. 508.—Female calf, born May 7. Attendant gives a history of digestive disturbance (scours) beginning a few days after birth and lasting 3 weeks. When first seen June 3, the calf was emaciated, weighing 75 pounds. The respirations were rapid and shallow. Both nostrils were soiled with whitish, mucoid masses. The right ear was held low and was discharging a viscid, pus-like matter. The temperature fluctuated between 39° and 40°C. until June 7, when it was killed.



TEXT-FIG. 3.



TEXT-FIG. 4.

TEXT-FIGS. 3 and 4. Dorsal and ventral aspects of lungs of Calf 508. For explanation of the shading see Text-figs. 1 and 2.

The autopsy showed the usual lung involvement (Text-figs. 3 and 4). Three types of lesions are present: (1) the focal necroses; (2) a firm hepatization with slight enlargement of the cephalic and ventral lobes, which are furthermore permeated with grayish foci 1 to 2 mm. in diameter; and (3) a more recent stage, characterized by a uniformly dark red airless condition. The affected lobes are furthermore variegated by air-containing territories. The lesion referable to the right ear was not traced, owing to the injury inflicted by the blow necessary to stun the animal. A small cavity was found in the temporal bone filled with a puriform liquid which may have been associated with the lesion. Films from this fluid contained numerous polynuclears and several kinds of bacteria (diplococci, bipolar and minute bacilli). Cultures from various regions of the diseased lungs were made. In all a variety of bacteria developed, among them *B. pyogenes*. *B.*

actinoides was detected in all serum cultures. In two tubes the colonies of this bacillus were present in large numbers and among them scattering liquefying colonies of *B. pyogenes*. Pure cultures of *B. actinoides* could not, however, be obtained from these tubes. The sections of material fixed in Zenker's fluid did not show any details differing from those already described.

As an illustration of the condition of the lungs of an animal surviving this type of pneumonia, the following case from the first outbreak is of interest.

No. 6.—This calf was nearly 7 months old when killed. It was born in June, 1916. When received the calf was normal as to temperature, respiration, and pulse. It was thin and hide-bound, but not unusually weak. No early history was obtained. It was killed because unthrifty. The disease was restricted to the right cephalic lobe of the lung. It was adherent to surrounding structures by easily broken fibers. One-half of the lobe is permeated with yellowish white sacs containing a thick creamy fluid. The other (caudal) half of the same lobe presents a grayish mottling of regular pattern. The air tubes of the entire lobe are surrounded by broad, whitish bands, or zones, and contain viscid, glairy molds. The trachea contains a large amount of a viscid, flour-paste-like whitish matter. In sections of the affected lobe the air tubes stand out prominently as thickened tubes. The thickening is due to a broad envelope of plasma cells and new connective tissue around them, while what is left of the parenchyma is slightly emphysematous and contains scattering desquamated alveolar cells (Fig. 13). A number of partly occluding ingrowths into small air tubes are made up largely of an endothelial cell type with an admixture of polynuclears. In a mediastinal lymph node, associated with the diseased lobe, the normal lymphoid cells are almost entirely replaced by the plasma cell type. Cultures from the various lobes developed a rich growth of a bacterium which agreed in cultural characters with the bipolar type of organisms (*B. bovisepticus*). Its virulence towards rabbits was very low. At this time *B. actinoides* was not yet known to the writer. The lesions were ascribed to *B. bovisepticus*.

After an interval of 6 months following the second (1919) outbreak a sporadic case of pneumonia appeared, which is of importance in several directions.

No. 544.—Female calf, born Oct. 6, 1920. It was reported sick by attendant Nov. 18 and transferred to the Institute next day. The animal was emaciated and very weak, unable to stand. The respirations were rapid and shallow. The buttocks were soiled with feces. Temperature on Nov. 19 was between 41° and 41.8°C. during the day. The animal refused all food. Early Nov. 20 the attendant found the calf panting and grunting. It died soon after and was autopsied at 10 a.m.

Besides the extensive pneumonic changes, the only noteworthy lesion is a septic condition of the stumps of both iliac arteries. The lumina are patent and the intima is coated with a thin, grayish, pultaceous layer not removable by washing. One artery contains a grayish yellow, cylindrical thrombus about 2 cm. long. The lungs are extensivcly hepatized. Even the left caudal lobe is nearly one-half solidified. The cephalic and ventral lobes of both sides have dimensions about twice those of the normal collapsed lung. The hepatization is smooth, grayish red, excepting where the cut bronchi exude whitish, pasty masses. The hepatization of the azygous and the caudal lobes is of a dark red color, less firm. The pleura is free, adhesions absent. Necroses are not detected in any lobe. Both main bronchi contain flakes of thick, glairy matter filling the mouths of many branches. The trachea contains many roundish masses of the same exudate, pea size and coated with froth. Tubes inoculated from various affected lobes, about twenty in all, develop, with one exception, into pure cultures of *B. actinoides*.

The nature of the pathological process was not made clear in the sections examined. The invasion of the epithelium of the ultimate bronchioles shown so clearly in No. 440 could not be seen. The air tubes were plugged with cell masses extending into them from the alveolar ducts. The cells consisted largely of polynuclears, feebly tinted necrotic cells, and cells resembling polynuclears in staining but with roundish nuclei. Throughout the affected lung tissue cells of endothelial and plasma type were abundant and intermingled with the others. Owing to the congested condition of the capillary network their relation to the alveolar walls was not clear. Cloud-like masses of minute bacilli were present among the cell masses and resolved with difficulty into their elements.

To bring this case into relation with those already described it may be assumed that the infecting agent was introduced and disseminated through the lungs in such large numbers and deposited in so many places that the process did not have time to reach the stage of focal necrosis before the calf died.

To the information gained by the two groups of cases in the same large herd may be added some data obtained from the autopsies of calves used for producing small-pox vaccine in the vaccine laboratory of the Massachusetts State Board of Health in Boston. During the year 1908 a small number of pneumonic lungs were found in calves killed after the vaccine had been removed. They weighed between 150 and 200 pounds.

No. A.—Both cephalic lobes are pneumonic. The affected tissue is flesh-red with lighter mottling or else simply collapsed. The air tubes contain molds of creamy pus. Sections show the stage of general polynuclear infiltration of parenchyma and the filling up of minute air tubes with the same kind of cell masses.

In one section there is a cellular irruption into an air tube and in the proliferated mass are colonies of minute bacilli.

No. B.—Both cephalic and ventral lobes are consolidated, and there is also a large focus in the left caudal lobe. Involved tissue is grayish red to grayish yellow, delicately and regularly mottled. The air tubes are distended with whitish, viscid plugs. The process in the focus in the caudal lobe is freshest, that in the free tips of the ventral lobes oldest. In general the histological picture is that of collapse, and infiltration with polynuclears. In one section there are two contiguous foci of cell exudation and hemorrhage undergoing necrosis. Many colonies of fine bacilli are in the periphery of these foci (Fig. 10). These cases, as far as the information goes, suggest an underlying process like that initiated by *B. actinoides*.

The Pathogenic Action of Bacillus actinoides.

The bacteriological study of spontaneous cases pointed to *Bacillus actinoides* as the primary inciting agent of the bronchopneumonia with *Bacillus pyogenes* and more rarely, in older calves, *Bacillus bovisepticus* settling down in the occluded air tubes and necrotic lung tissue. The next step was to determine whether cultures of this bacillus, under suitable conditions, could produce lesions like those found in spontaneous cases. In the following pages is given a brief statement of observations made on inoculated calves, since small mammals had thus far shown themselves quite insusceptible. Some of the calves were inoculated subcutaneously, some intravenously, others into the trachea. The area of the operation was shaved, cleansed with water and alcohol, and painted with tincture of iodine. In the intratracheal injections, a small incision was made through the skin over the trachea before inserting the needle.

No. 467.—Bull calf, born Jan. 21, 1920. Dam had placenta retained at birth and swab from uterus as well as agglutination test was positive for *B. abortus*. Temperature, taken twice daily, fluctuated between 38.5° and 39°C. until Feb. 3, the day of inoculation.

The turbid condensation water of a horse serum culture of *B. actinoides* from Calf 462, 5 days old and under artificial cultivation since Jan. 7, was drawn off, making about 1 cc. in all. This was increased to 3 cc. by the addition of sterile bouillon. The fluid was injected into the subcutis in front of right shoulder. The temperature rose to 39.8°C. within 3 hours and remained at the same level next day. On this day a local swelling appeared over the point of inoculation about 1½ inches in diameter and ¼ to ⅜ inch thick. The temperature fell below 39°C. on Feb. 8. The swelling increased slightly.

On Feb. 19, a second inoculation was made, this time into a jugular vein with the same strain used for the first, subcutaneous inoculation. The condensation water was ground slightly in a crucible to break up the flocculi and calf serum water was added to dilute the suspension. The temperature rose to 40°C. within 3 hours and gradually fell to normal during the night. No further elevation occurred. The calf was killed Mar. 4. The weight had risen from 88 to 125 pounds. The local swelling was about the size of a small hen's egg. The calf was stunned with a heavy blow, a clamp quickly placed on the trachea, and the neck vessels were severed. During this operation the local swelling ruptured and several cubic centimeters of a thick, whitish mass, like flour-paste, were discharged. The autopsy showed normal organs, except for two very small foci of collapse, one in the right cephalic, the other in a caudal lobe of the lungs. The local swelling is adherent to the overlying skin, but readily dissected from the subjacent fascia. The under surface of the capsule is sprinkled with minute hemorrhages. It is 3 to 4 mm. thick, pearly white on section and enclosing a cavity partly filled with a soft, odorless mass like flour-paste. The inner surface of the capsule is dark red to hemorrhagic and delicately mammillated. The cavity has small recesses or pockets. The puriform contents are readily removed from the entire wall. The associated prescapular lymph node is about one-fourth larger than the opposite node and on section smooth and quite juicy. The contents of the abscess consist of cells, only a few of which retain the stain. These are mononuclear. Scrapings from the capsule show a mixture of polynuclear and mononuclear elements and occasional cloud-like masses of minute bacilli. Some of the mononuclear cells contain groups of minute bacilli. *B. actinoides* was recovered from the abscess both on horse serum and on agar plus blood. In one of the latter the growth appeared richer than usual and was found by inoculation into two guinea pigs and by microscopic examination to contain *B. abortus*. The presence of *B. abortus* is accounted for by the infection of the dam which had been demonstrated by suitable tests after the birth of the calf.

No. 336b.—Bull calf, born Feb. 28, 1920. On Mar. 9, it weighed 111½ pounds. A horse serum culture from Calf 462, 5 days old, was used. The condensation water, which contained a dense suspension of flocculi visible to the naked eye and shown to be pure by the microscope, was removed to a sterile tube, 5 cc. of bouillon were added, and the whole was thoroughly shaken. The flocculi were still visible after the shaking. The suspension was injected into a jugular vein at 4 p.m. The temperature rose from 38.8° to 40.6°C. in 5 hours and gradually dropped to 38.9°C. during the night. The calf was kept under observation for 36 days. During this period the temperature remained between 38° and 39°C. and the calf's general condition was normal. On the 36th day it weighed 145 pounds and was sold for slaughter.

No. 479b.—Bull calf, born Feb. 26. Inoculated on Mar. 9, when 12 days old. A first culture from the local lesion of Calf 467 on agar plus blood, 5 days old, was used. The surface growth was transferred with a platinum loop to 5 cc. of bouillon until a fine suspension was produced. This was made up of excessively fine

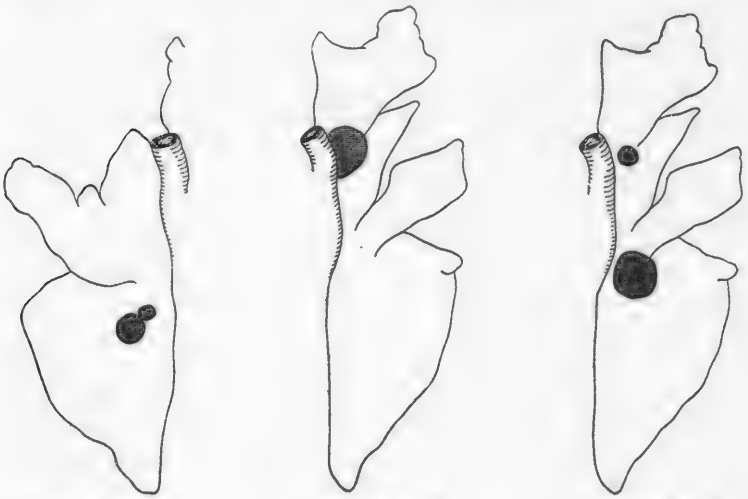
granules when viewed in a strong light. It was injected into a jugular vein. Calf weighed at this time 97½ pounds. The temperature rose from 38.7° to 40°C. within 5 hours. It was only a few tenths above normal next day. The calf continued normal in condition. The temperature remained normal.

On Mar. 24, 15 days after the intravenous injection, a second inoculation was made, this time into the trachea. The condensation water of three horse serum cultures of *B. actinoides*, 4 days old, from Calf 467 was removed to a sterile tube and enough bouillon added to make 12 cc. in all. 5 cc. of this were injected. Within 24 hours a swelling appeared over the trachea, of undefined borders, probably 6 inches long and 2 inches broad. After several days this swelling was somewhat reduced and was now found to be made up of two indurations, one over the point of injection, the other lower down at root of neck. Apr. 5. The right leg was shown to have restricted motion and to be painful when handled. Apr. 15. The right shoulder appeared a trifle larger than the left and tender. The lameness increased. The calf, showing no general symptoms, was killed Apr. 20, 27 days after the intratracheal injection. The only lesions found were the local swellings. One about 2 inches in diameter is situated just caudad of the larynx; a second, 4 inches in diameter, just caudad of the first. Both are firm, tense, slightly fluctuating. They are covered by muscles of the neck and attached to the cervical portion of the thymus. Both are sacs with walls 5 to 10 mm. thick, smooth interiorly, and filled with a soft odorless mass, resembling cottage cheese. They communicate with each other through a narrow opening. The contents consist of lymphocytes and polynuclears, the former greatly predominating. Both kinds of cells largely refuse the stain. There are also filamentous masses, probably necrotic fibers. In the larger abscess a strip of necrotic tissue was found in the caseous contents about 4 inches long, representing the remnants of a vessel about 2 mm. internal diameter. After washing away the pus the external surface was found covered with small papilla-like projections.

On the prominence of the right shoulder joint there is a swelling about the size of an egg, easily dented by pressure. The tumor is situated between the tendinous ends of the muscles inserted in the scapula. There is marked thickening of the intermuscular fascia radiating from the capsule of the tumor to a depth of several inches. On incision of the thick capsule, an irregular cavity is exposed, with contents mucoid, whitish, quite unlike those of the tumors over the trachea. However, films show the same cell elements, the difference being due to the advanced necrosis of the contents of the other abscesses.

Sections of the abscess walls show a mass of muscular and connective tissue with marked hyperplasia of the connective tissue and some fibrin. There is also a general infiltration with cells of endothelial character. The pus attached to the inner wall consists wholly of cells of endothelial type. Some of these are filled with minute bacilli. There are also clouds of free bacilli feebly stained and easily overlooked. Cultures prepared from both abscesses over the trachea develop into pure growths of *B. actinoides*. No cultures were made from the abscess on the shoulder. The respiratory tract as well as the remaining viscera was normal.

No. 495b.—Female calf, born Apr. 15, 1920. On May 11, 10.30 a.m., when 26 days old and 88 pounds in weight, it was inoculated into the trachea. The condensation water of six horse serum cultures from Calf 479b, 5 days old, was drawn into a fresh tube and 6 cc. of bouillon were added, making 13 cc. of heavily clouded fluid. 8 cc. of this were injected. Animal coughed occasionally after



TEXT-FIG. 5. TEXT-FIG. 6. TEXT-FIG. 7.

Effect of the intratracheal injection of cultures of *B. actinoides*.

TEXT-FIG. 5. Necrotic focus in left caudal lobe of Calf 520.

TEXT-FIG. 6. Similar focus in right cephalic lobe of Calf 474b.

TEXT-FIG. 7. Two necrotic foci, one in cephalic, the other near root of right ventral lobe of Calf 495b.

the operation. The temperature rose about 1°C. during the day and was down again the next day. 3 days later the temperature reached 40°C. and fluctuated between 40.2° and 40.7°C. for 6 days, then dropped to 38.3° to 38.7°C. The calf weighed 99 pounds on May 27, having gained 11 pounds in 16 days. It was killed on this day.

The digestive tract is normal throughout. In the respiratory tract there is a bit of semitranslucent mucus on one vocal cord. In the lower trachea there is a similar mass, pea size, made up of alveolar epithelium and polynuclear leucocytes. There are two groups of focal necroses in the lungs (Text-fig. 7). On the dorsal ridge of the right caudal lobe, near root of the right ventral, there

is a small, whitish, opaque, subpleural mass. On palpation, other firm nodules are felt embedded, about half a dozen in all. On section the contents show as thick, cheesy cores in dense capsules. The tissue between them is still air-containing. There is no fresh pneumonia or collapse around them. A similar cheesy nodule is in the right cephalic lobe, embedded in emphysematous tissue. Besides these two necrotic foci there are several collapsed lobules in different lobes. Lymph nodes of thorax are normal. There are 10 cc. of clear fluid in pericardial sac. Both kidneys are spotted everywhere with whitish areas, discrete and confluent, occupying fully one-half of the total cortex. These spots are the bases of cones of fan-shaped outline on section, extending to the medulla. The substance of these foci is glistening, smooth, almost like cartilage in appearance. Urine taken from the bladder soon after death is very pale, clear, faintly alkaline, specific gravity 1,004. Boiling after adding 1 to 2 drops of acetic acid yields a very faint cloud. The liver is slightly fatty.

Sections of the necrotic foci in the lungs show centrally a nucleus of dead lung tissue in which the alveolar structure is still preserved (Figs. 15 and 16). These nuclei are 1.5 to 2 mm. in diameter. In the central core the alveoli are nearly empty. In the peripheral zone, they are filled with a fine granular material in which many cells are embedded. Immediately surrounding the necrotic center is a broad zone in which the lung structure is occasionally seen, but is chiefly replaced by cellular debris embedded in a granular matrix. Dense groups of minute bacilli are in the outer layers of this zone where many alveolar cells are still recognizable. Outside this zone is a layer of fibroblasts and newly formed capillaries. The whole is enclosed in a mantle of lymphoid cells, occupying the meshes of the compressed lung tissue. In the vicinity of the necrotic focus there are zones of lymphocytes around the ultimate and next larger bronchioles, associated with collapse and broadening of alveolar walls through cell infiltration. In some air tubes small groups of polynuclears are seen. Sections of the kidney lesions show interstitial hyperplasia with shrinkage and disappearance of glomerular tufts, dilatation of some convoluted tubules and disappearance of others. Cultures made from the necrotic lung foci both on horse serum and blood agar developed the characteristic appearances of *B. actinoides*. Only one contained also a fine filamentous growth, probably a streptothrix. All of sixteen cultures prepared from kidney tissue remained free from growth.

No. 474b.—Black and white bull calf, born Apr. 21, 1920. June 3. The calf weighed 135 pounds. The condensation water of three horse serum cultures, 4 days old, showing a dense crop of flocculi was withdrawn into a sterile tube, about 4 cc. in all. This was injected into the trachea, and without removing the needle, about 6 cc. of sterile salt solution were injected through it. After the calf had been placed on its feet it coughed some, ejecting a fine spray. Following the injection the temperature rose about 1.5°C. It was normal next day. The calf was killed June 21, 18 days after the inoculation. The autopsy showed normal conditions with the following exceptions. In the abdominal cavity there are

about 50 cc. of a clear, slightly yellowish fluid which coagulates into a jelly-like mass on standing. Between the vocal cords, along the trachea, and at the root of the right supernumerary bronchus are small masses of a semi-opaque, mucoid substance, consisting of polynuclears, alveolar, and endothelial elements embedded in mucus. The lungs show quite generally over all lobes scattering, very small, dark reddish collapsed or pneumonic foci from mere points to 1 mm. in diameter and of various shapes. In the right cephalic lobe near root is a consolidation about 3 cm. in diameter, but more or less squarish and extending through depth of lung tissue (Text-fig. 6, page 457). It feels lumpy. On section the lumps appear as spherical, firm, grayish foci, about 1 cm. in diameter and centrally necrotic. There is congested lung tissue between them. Each focus is made up of a very firm, almost cartilaginous capsule. Within is a viscid, very thick, whitish mass, which in the larger foci contains in it a more grayish, spongy nucleus found to be necrotic lung tissue. The layer around this contains many mononuclear elements whose cytoplasm, in many cells at least, is filled with fine rods. Masses of similar rods are free in the stained film. The necrotic masses are odorless. Cultures made from the necrotic foci on horse serum showed the characteristic flocculi in 3 days. All contained in addition molds with delicate mycelium. Pure subcultures of *B. actinoides* were obtained, however, from surface colonies. Cultures on blood agar were also prepared. Some were pure growths of *B. actinoides*, others contained one or several other types of colonies. At the same time, cultures were prepared from the air-containing, normal lung tissue by introducing into culture tubes small bits of lung tissue. Molds, liquefying colonies, and several other types of colonies appeared in the tubes.

No. 504b.—Black and white female calf, born June 10, 1920. Weight, June 21, 110 pounds. On June 28, the condensation water of six serum tubes of *B. actinoides* from Calf 474b, 3 days old, was brought together in a sterile tube. The heavily clouded fluid, 5 cc. in all, containing flocculi was injected into the trachea at 3 p.m. The temperature rose about 1.5°C. within 4 hours, then slowly subsided. No abnormal conditions appeared, and it was killed 23 days after inoculation. At this time it weighed 136½ pounds. No lesions were found in the respiratory tract or elsewhere.

No. 520.—Guernsey calf, male. Born Oct. 8, 1920. Weight on Oct. 22, 83½ pounds. On Oct. 27, four horse serum tubes inoculated from cultures of Calf 474b, 4 days ago, were used for intratracheal injection. The condensation water was drawn up into a sterile tube, a little salt solution being used to wash it out more thoroughly. 5 cc. of a moderately clouded fluid were collected in this way. This was injected into the trachea at 10.30 a.m. Before removing the needle, 10 cc. of sterile Ringer's solution were injected. The temperature rose from an average of 38.6° to 41°C. 7½ hours after the injection and gradually fell during the night—the temperature being taken every 2 hours—to 38.8°C. next morning. Following the injection the calf began to appear depressed, the respirations were slightly irregular, about 90 at 2.30 p.m. Attendant noticed a slight chill between 4.30 and 5.30 p.m. The temperature did not go above normal again and the

calf, showing no signs of any disturbance thereafter, was killed Nov. 12. The weight on this day was 96 pounds. The organs were normal with the following exceptions.

Beginning about 5 cm. below lower margin of the larynx, the mucosa covering six intercartilaginous spaces of the trachea is dark red over each space. There are no signs of swelling. In the left caudal lobe a firm mass is buried within air-containing, slightly emphysematous lung tissue (Text-fig. 5, page 457). It is about 1 cm. in diameter. Soft, smooth, flour-paste-like material oozes out from a slight incision. The mass is placed in Zenker's fluid. A film of this thick mass shows necrotic cells and no bacteria. There is a second 2 to 3 mm., firm, yellowish white focus near the first. It contains a nucleus of necrotic lung tissue, enveloped in the same material found in the larger focus. Films of this contain large numbers of minute bacilli among cells of endothelial and leucocyte type which hold the stain as if still living. Cultures were not prepared from this case, owing to the scanty material and the need for histological examination of the lesions. This did not, however, show more than has been given from the other cases in which intratracheal injection of *B. actinoides* produced focal necrosis. The central nucleus of the necrotic tissue was surrounded by a layer of polynuclear leucocytes and outside this a connective tissue capsule was forming. Between these two layers, within a narrow zone, colonies of minute bacilli were abundant. Many groups of bacilli were within the cytoplasm of cells.

The results of the inoculation of cultures of *Bacillus actinoides* may be briefly summarized. The subcutaneous injection leads promptly to a large swelling which becomes very firm. After several weeks the mass of the swelling becomes necrotic, the contents caseous, and a thick, firm wall forms with hyperplasia of the connective tissue in the immediate environment of the focus. Softening of the capsule, ulceration of the overlying skin, and discharge outward take place within 4 weeks. In several cases, in which the injections were into the trachea, a small amount of the culture fluid entering, accidentally, the tissues overlying the trachea led to large abscesses. The swelling is primarily due to an increase of mononuclear, endothelial-like elements, with later invasion of polynuclears. The final product is a soft mass, like cottage cheese in appearance, consisting of small lumps, the whole embedded in a thick mass, smooth, glistening, like flour-paste in consistency. In one abscess the remnant of a vessel, 4 inches long, was embedded in the mass. Clouds of minute bacilli are found in the zone outside the necrotic mass where the cellular elements are still stainable. In one case (No. 479b) a second (metastatic) abscess developed at some distance from the site of inoculation.

Injection into the circulation in one case failed to produce any lesions. The second case was sold in excellent condition, probably without lesions. Injection into the trachea failed in two out of five cases to produce any appreciable changes in the lungs. In the remainder there developed small necrotic foci, single or multiple, identical both macroscopically and microscopically with those occurring in spontaneous cases.

The distribution of the induced lesions did not agree entirely with that of spontaneous cases, thus meeting in part the possible objection that the lesions might have been due to naturally acquired infection. The difference in localization was probably due to the fact that the calves were lying on their backs when the culture fluid was injected. This abnormal position may have led to a different drainage of the injected fluid from that occurring in spontaneous cases.

In one instance (No. 495b) the lung lesions were associated with multiple focal scleroses of the kidney cortex.

The culture used was obtained from Calf 462 and passed in succession through Nos. 467, 479b, 495b, 474b, and 520. This was done with the expectation that the virulence would maintain itself. From every one of the above, excepting No. 520, pure cultures were recovered. None were made from the last case.

It will be noted that the intratracheal inoculations produced what was called above the first stage of the disease only. The lesions were restricted in extent and the secondary lesions due to dissemination of the bacilli from the primary necrotic foci did not take place. Perhaps some special depressing conditions may be needed to continue the disease into the clinical stage.

In contrast to the very acute, both destructive and tissue-stimulating action of *Bacillus actinoides* in the subcutis of calves is the following negative action on one sheep.

No. 164.—Barren ewe. On May 11, 1920, the same suspension of cultures from Calf 479b used on No. 495b was injected under the skin in front of left shoulder. Neither local nor thermic reaction followed. The experiment was closed June 1.

Correlation of the Pathological and Bacteriological Data.

The foregoing observations and experiments demonstrate the existence of a specific bronchopneumonia causing mortality in calves chiefly in the 2nd and 3rd months of life. In animals which die within the 1st month other agencies due to fetal conditions and to infections acquired during or soon after birth may involve the lungs. Surviving cases affected with a chronic pathological condition of one or more lobes, characterized by purulent bronchial exudation, abscess formation, and fibrosis may be met in the 4th to the 6th month or even later. As a rule, the disease invades both lungs symmetrically. The parts affected first are the smaller cephalic and ventral lobes, more particularly the dependent portions (Text-figs. 1 to 4). With the progress of the disease the involvement moves upwards towards the dorsal border of these lobes and backwards into the azygous lobe and the caudal lobes. Death takes place when one-half of the latter have become airless. The pleura is involved only where necroses extend to the surface. Here adhesions to surrounding structures form from the capsules of the resulting abscesses.

Several kinds of lesions are presented in the ordinary acute case. The distal two-thirds of the cephalic and ventral lobes are, as a rule, considerably enlarged beyond the normal collapsed state, very firm, dark or light reddish in color. Regularly sprinkled in this ground are grayish, 1 to 2 mm. areas. The proximal third of the same lobes and the affected regions of the caudal lobes are but little enlarged, uniformly dark red, less firm, and without the grayish mottling. Careful search, by manipulation if necessary, reveals scattering firm masses, sometimes deeply embedded, more commonly extending to one or both lung surfaces. They vary from 2 to 10 mm. in diameter. Several may coalesce. They consist of a pearly, dense capsule containing necrotic lung tissue, enveloped in a layer of viscid pus. These sequestra are with rare exceptions located in the cephalic and ventral lobes. They are probably among the oldest lesions. They and the mottled pneumonic regions which are always associated with them are either developed at the same time or else the necroses are the source of the infection which produces the pneumonic condition. The smooth dark red pneumonia is secondary to the other lesions,

probably through the agency of aspirated purulent exudates. The distribution and extent of the three conditions are shown in the text-figures. The pneumonic condition is usually not universal in the affected lobes. Small or large air-containing territories may occur in them, chiefly along the free margins of the lobes. The one universal characteristic lesion is the filling of the air tubes of the affected lobes with a thick, viscid, glairy, white, mucopurulent matter.

The microscopic characters of the different stages are fairly well definable. The formation of the necroses (Figs. 12, 15, and 16) has not been traced, owing to lack of material in the early stages. Associated with or following these, there is a filling up of the alveoli with several cell types to form the mottled pneumonic territories. At the same time the ultimate bronchioles and alveolar ducts may become involved. The entire parenchyma becomes filled with mononuclear elements, probably a mixture of alveolar cells, endothelial and lymphoid cells. The polynuclear leucocyte is absent in this stage. The smooth, fresher pneumonic condition which develops later is due to partial or complete filling up of alveoli and bronchioles with polynuclear leucocytes. In these the epithelium remains intact. With this stage there appears the zone of plasma cells around the air tubes filled with cell debris (Figs. 7 and 13). Numerous polynuclear leucocytes are found moving outward through the epithelium into the lumina. This accumulation of plasma cells is probably due to the cell debris in the lumina of the air tubes. They are present after a certain time whether one or several species of bacteria are at work. That they are the result of the stimulus exerted by toxins and other products absorbed from the disintegrating cellular plugs in the lumina seems to be at present the most plausible explanation of their presence. The minute grayish dots permeating the older pneumonic lesions, which are so striking in the fresh lung of most but not all cases, have not been associated definitely with any microscopic details. They may represent the earlier proliferative lesions in the ultimate bronchioles in some cases and the gathering of plasma cells in later stages.

Before summarizing the results of the bacteriological studies it should be stated what is apt to be forgotten in an interpretation of results, that the normal lungs of calves, and other species as well,

contain a considerable variety of living microorganisms. When bits of normal lung tissue are placed in culture tubes, growth appears quite invariably. Spore-bearing bacilli, various kinds of molds, and streptothrix are among the commonest forms detected. In the isolation of *Bacillus actinoides*, on agar, bits of lung tissue are essential to growth. On coagulated serum tissue is not necessary. It is somewhat surprising that so many cultures obtained from bits of diseased lung tissue were pure cultures of *Bacillus actinoides*. It would seem as if under the influence of the violent tissue reaction the banal forms found in normal lungs are largely destroyed.

The bacteriological examination has shown a variety of results, depending on the stage of the pneumonic process, the rapidity of its development, and whether the animal died or was killed. In the 1919 to 1920 epidemic, twelve cases were investigated. *Bacillus actinoides* was the only cultivable organism present in five of these. It was present but associated with other pathogenic species in four more. It was not detected in the remaining three. *Bacillus pyogenes* was present in seven cases but not in pure culture. *Bacillus bovisepticus* was present in three or four cases, always with a variety of other species. Of the five cases in which *Bacillus actinoides* was exclusively present, two had died and three were killed. Of the four in which it was associated with other species, three were killed and one died. Of the three in which it was missed, two died and one was killed. A study of the brief protocols suggests that *Bacillus actinoides* was missed through overgrowth with other species in certain very acute dead cases and in the more chronic surviving cases.

In the tissues of the diseased lungs *Bacillus actinoides* is found in the peripheral layer of lung sequestra, in colonies in the cell masses occupying alveoli and alveolar ducts (Figs. 8 to 11), and in the proliferated epithelium obstructing the bronchioles less than 0.1 mm. in diameter (Figs. 1 to 7). This latter process which is rather unique and which has not thus far been described in the pathology of pneumonias was especially well brought out in sections of No. 440, but it could be detected in most other cases after more or less searching. In the invaded epithelium *Bacillus actinoides* appeared with the sheath or capsule around it. In other situations it was usually free from capsules and appeared in dense, cloud-like colonies. It should

be stated here that, with rare exceptions, air tubes over 0.1 mm. in diameter had their epithelium intact. The cell masses with which many were filled—the glairy, mucopurulent contents—consisted of cell debris moving up from the ultimate bronchioles and the parenchyma.

After injection of pure cultures into the trachea *Bacillus actinoides* has thus far produced only the early stage of necrosis (Text-figs. 5 to 7 and Figs. 15 and 16). The diffuse secondary and later lesions characterizing the spontaneous fatal and very sick cases killed were absent. The relation of *Bacillus actinoides* to the primary necrotic lesions is thus placed beyond doubt. That it is also responsible for the general pneumonic involvement is not proved by experiment but made highly probable. Neither *Bacillus pyogenes* nor *Bacillus bovisepticus* is responsible, since these easily cultivated bacteria were absent in five advanced cases. Considerations based on bacteriological and histological studies are sufficient to throw them out of the other cases in which they were found, except in the rôle of continuing a disease fully under way by multiplying in the necrotic tissues and the bronchial cell debris. If *Bacillus pyogenes* and *Bacillus bovisepticus* are not responsible for the diffuse pneumonic lesions, then either *Bacillus actinoides* or else some non-cultivable, unrecognized microorganism is. The writer has throughout assumed that *Bacillus actinoides* is the sole responsible agent.

A study of the mode of response or reaction of the tissues to *Bacillus actinoides* has not come within the scope of the present investigation. The finding of some small susceptible species is necessary to provide material for such study. In general the reaction in the lungs is at first associated with mononuclear cell types, either endothelial or lymphoid or both. In later stages polynuclear cells completely dominate the process. This change may be due to an immunity reaction. Many things point to this explanation. Thus the later smooth pneumonic condition is always associated with the polynuclear cell type. In those animals in which the process halts with the necroses, the remaining parenchyma contains only polynuclears in focal distribution. Moreover, *Bacillus actinoides* is only very rarely detected with the microscope in the lesions associated with polynuclear cells, although cultures reveal its presence. This rarity suggests that the active stage of multiplication is over.

The various factors entering into the process such as quantity and dissemination of the original infecting material, relative susceptibility of the host, and the rapidity with which immune forces are called forth determine the extent and rapidity of the early, usually necrotic changes and the promptness of dissemination of the virus from such necrotic foci over new territories. Added to these factors are species of bacteria, chiefly *Bacillus pyogenes* and *Bacillus bovisepticus*, which may graft themselves on the diseased tissues. In thus restricting *Bacillus bovisepticus* to a secondary position, the author does not imply that there may not be virulent races of *Bacillus bovisepticus* capable of initiating outbreaks of pneumonia.

Aside from Case 6, no detailed description of the cases occurring in the 1917 outbreak, which was the basis of an earlier publication,[1] has been given. This group of cases had not been worked up with so much care and in such detail partly because the very first case which came to autopsy yielded pure cultures of *Bacillus bovisepticus* and the developing epizootic was considered due to this bacillus. With the succession of cases, the appearance of a polymorphic bacillus and its association with a closely resembling species, *Bacillus pyogenes*, rendered orientation very difficult until a pure culture of *Bacillus actinoides* was made to grow indefinitely in subcultures and its forms clearly differentiated from *Bacillus pyogenes*. A repeated study of the accumulated data has shown such a parallelism between the 1917 and the 1919 cases in gross and minute anatomy and histology of the affected lungs and in the bacteriology that any review of these early cases would be in the main a repetition of what precedes. The underlying etiological factor and the processes it initiates are the same in both groups of cases.

A study of the epidemiology of the pneumonia due to *Bacillus actinoides* needs much additional material. The source of this microorganism is not yet defined. It will, however, be safe to regard partly recovered older animals of the same species as the purveyors of the virus. The termination of certain lesions in necrosis followed by copious discharge from the resulting abscesses opens the way for the virus outward. It is probable that unrecognized cases occur in which the process does not go beyond localized necrosis of small territories. Such cases may serve in maintaining the mild disease

until the cold season favors the secondary extension of the lesions and brings acute clinical cases to the surface. This view is supported by the production of necroses experimentally in calves without any appreciable rise in temperature or other suspicious deviations from health.

Owing to the meager descriptions given in reports of outbreaks of pneumonia in calves, it has been impossible to identify this form of bronchopneumonia in earlier writings. An attempt was made in the article on *Bacillus actinoides*[1] to correlate the latter with Lignières' actino-bacillus which produces subcutaneous and other abscesses but not pneumonia. A continued study of *Bacillus actinoides*, however, does not make the relationship seem any more real. It remains to study calf pneumonias in both anatomical and microbiological directions afresh, since the existence of several etiological types of lung disease is probable. The anatomical and histological study of these pneumonias is important, since information gained in this way tends to restrain hasty conclusions concerning the etiology where several different species of bacteria may find opportunity for multiplying in the lungs in the course of the disease.

CONCLUSIONS.

A bronchopneumonia of calves in the early months of life is described and its etiology associated definitely with a minute bacillus, *Bacillus actinoides*. *Bacillus pyogenes*, *Bacillus bovisepticus*, and, less frequently, staphylococci and streptococci may appear later in the affected lungs.

Subcutaneous injections of cultures of *Bacillus actinoides* produce large indurations ending in necrosis. Similarly intratracheal injections produce circumscribed necroses of lung tissue. The cultivation of *Bacillus actinoides* and its morphological peculiarities have been sufficiently described and illustrated in an earlier publication[1] to ensure success on the part of those who attempt to isolate it.

The writer is indebted to Dr. R. B. Little for assistance in bringing together the clinical data.

EXPLANATION OF PLATES.

PLATE 42.

FIG. 1. Section of lung tissue, Calf 440, showing dilatation of a small bronchus and complete filling up of the lumen with the characteristic exudate. The epithelium has disappeared from most of the circumference. The exudate is intimately associated with the subepithelial tissues. The parenchyma around the bronchus is partly collapsed, partly pneumonic. The capillaries are distended with corpuscles, and the alveoli are filled with cells of endothelial and lymphoid type. See Fig. 4 for magnification of a part of this exudate. × 240.

PLATE 43.

Both figures from Calf 440.

FIG. 2. An air tube about 0.1 mm. in diameter with ingrowth of cellular masses, which are filled with bacilli, as shown in Fig. 5. The section passes through a dividing bronchus, of which one branch is involved. Remnants of the epithelial cells are traceable in the irrupted mass which contains cells with round, pycnotic nuclei. × 240.

FIG. 3. Transection of an air tube about 0.3 mm. in diameter. The exudate is intimately connected with the subepithelial tissue of the bronchus in two places. Elsewhere the epithelium is still *in situ*. The exudate is of the same character as in Figs. 1 and 2. It is permeated with cloud-like masses of minute bacilli showing only as slightly darker patches in the photograph. × 310.

PLATE 44.

FIG. 4. A portion of the diseased bronchus of Fig. 1, enlarged × 1,000. The bacillar and capsulated or club-shaped forms are present, filling the cytoplasm of the cell mass which has nearly plugged the lumen of the small bronchus.

FIG. 5. A portion of the diseased bronchus of Fig. 2, enlarged × 1,000. The bacillar, cloud-like masses have filled the cytoplasm of the cells as in the preceding figure. The club-shaped forms are shown in the lower right-hand region in many cross-sections and rather sparsely elsewhere in the bacterial masses.

PLATE 45.

FIG. 6. From Calf 440. Section through a small bronchus, of which the epithelium along one margin (to the right in the figure) is still *in situ*. The other margin is obliterated by an ingrowth which nearly occludes the lumen. This ingrowth is densely permeated with *B. actinoides*. The bacilli appear chiefly as very short rods of irregular size and form throughout the section not taken up by the bronchial epithelium. × 1,000.

FIG. 7. From the same lung. The small air tube is nearly occluded by the mass of cells on the left. This mass is permeated densely with *B. actinoides* in bacillar form. In addition, larger circular areas, suggesting capsulated cocci, are sprinkled through the mass. These are identified as club-shaped forms of *B. actinoides* cut across. × 1,000.

PLATE 46.

FIG. 8. Section through the parenchyma of the lungs, Calf 440. The alveolus is filled with cells. In the center of the cell mass is a colony, or floccule, of *B. actinoides*. × 1,000.

FIG. 9. Two alveoli, each containing a floccule. The clubs are shown imperfectly in optical cross-section. × 1,000.

PLATE 47.

FIG. 10. Section from an old case, designated as Calf B on page 454 of the text showing two foci in which are cloud-like colonies of minute bacilli, appearing as irregular darker patches in the figure. × 1,000.

FIG. 11. Section from the lungs of Calf 440. In the upper left-hand corner is a large cloud-like colony of bacilli. Other colonies in the field are out of focus and appear as slightly darker patches. × 1,000.

PLATE 48.

FIG. 12. Sequestrum from the lung of Calf 462, slightly dislodged. The outlines of the alveoli may still be distinguished in the upper portion of the necrotic mass. The border of the wall or capsule above, which suggests epithelium in the figure, is compressed tissue made up of fibroblasts and plasma cells. × 25.

PLATE 49.

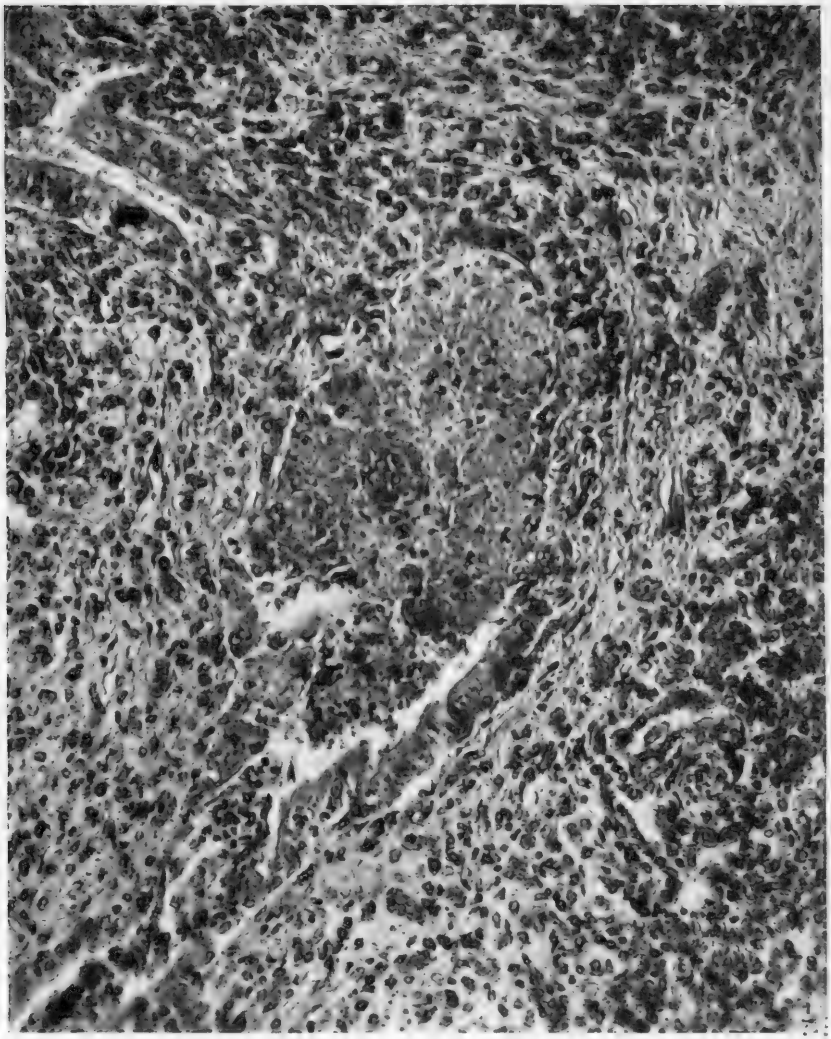
FIG. 13. Section from the lungs of No. 6. The more or less deformed bronchi are filled with polynuclear leucocytes and surrounded by a broad zone of plasma cells and fibroblasts. × 925.

FIG. 14. Section of lung tissue from Calf 446. This case is somewhat older than No. 440. Near the center of the figure is a more or less deformed bronchiole containing polynuclear leucocytes. The lung tissue in a broad zone around this tube is occupied by groups and masses of plasma cells and polynuclear leucocytes. × 925.

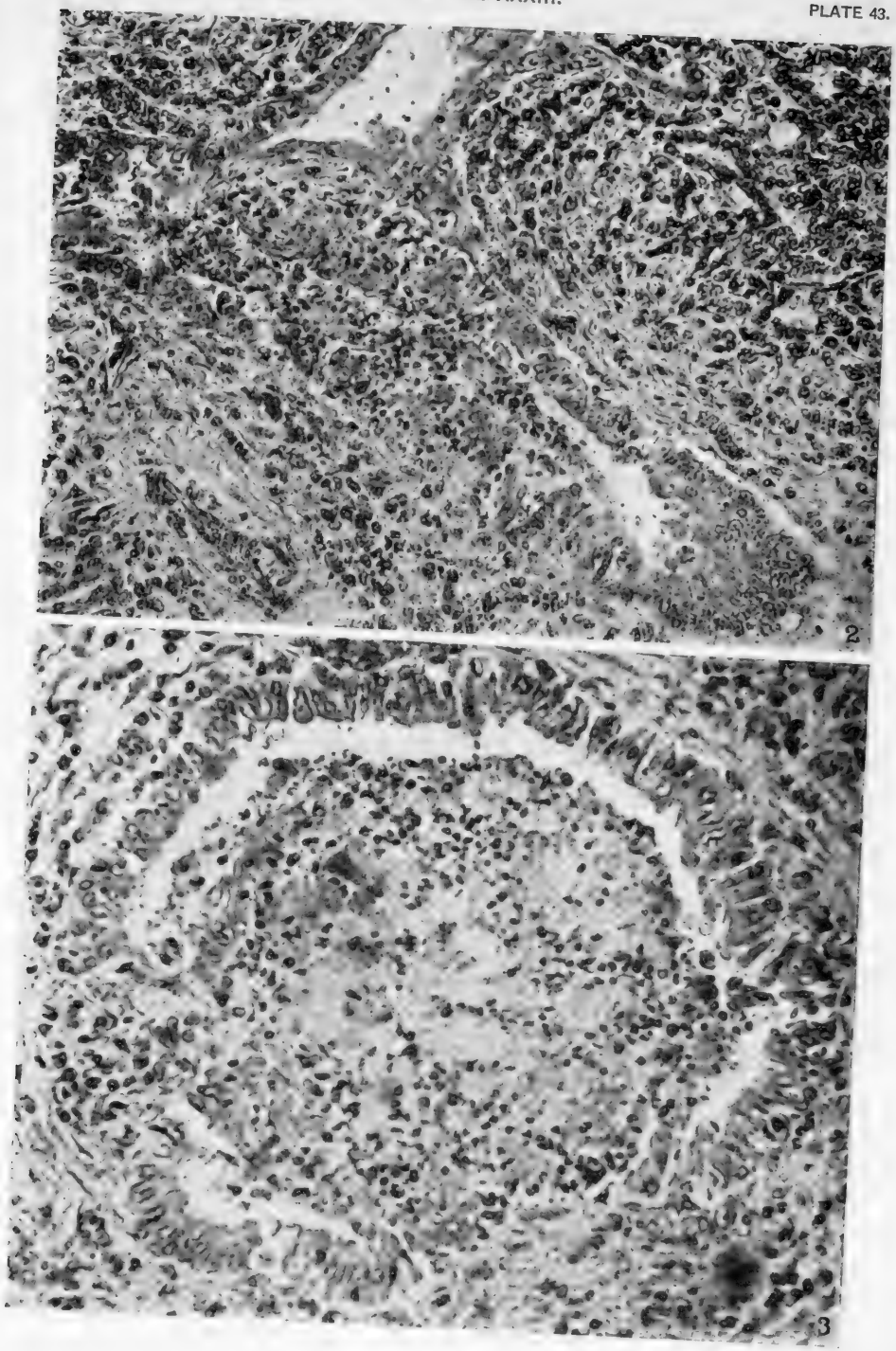
PLATE 50.

FIG. 15. Represents a necrotic focus from the lungs of Calf 495b (see Text-fig. 7) which received an intratracheal injection of *B. actinoides*. × 60.

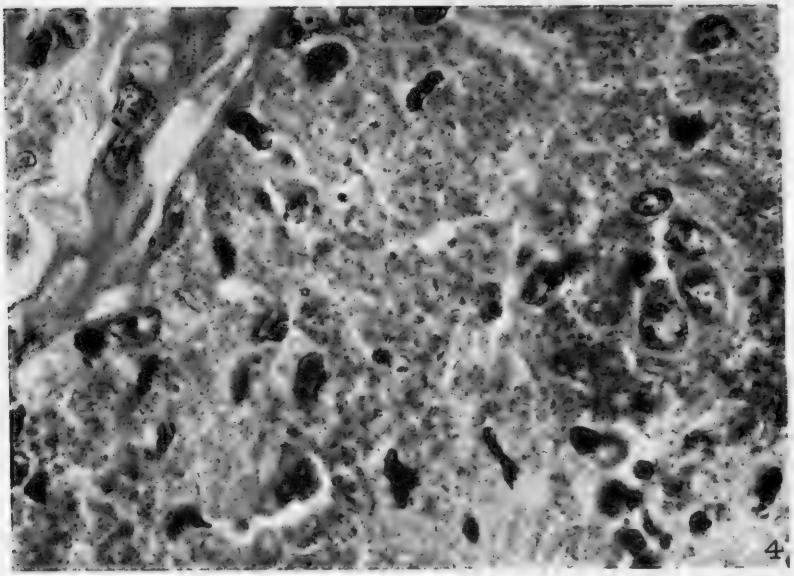
FIG. 16. Enlargement of the zone just outside the necrotic mass shown in Fig. 15. Irregular groups of *B. actinoides* occupy this zone. The large group on the right has only a few bacilli in focus, whereas the central group is fairly distinct. × 1,000.

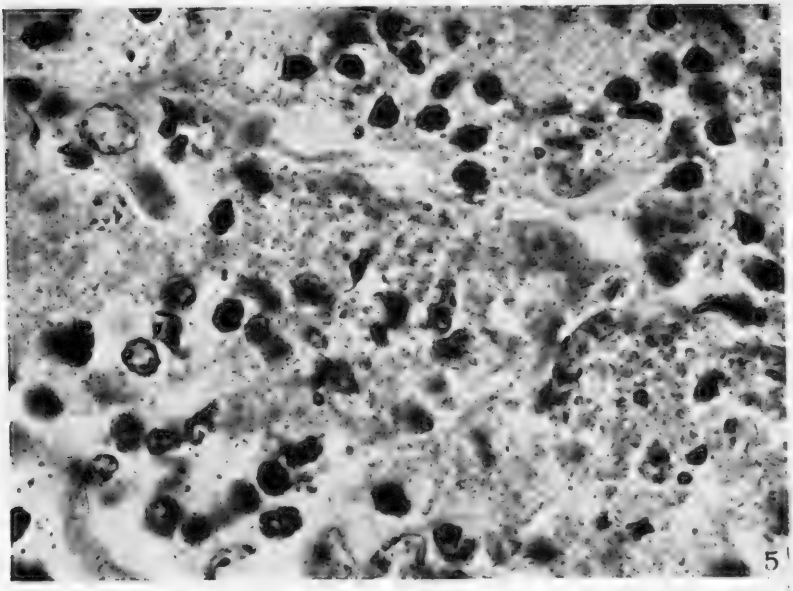


(Smith: *Bacillus actinoides.*)

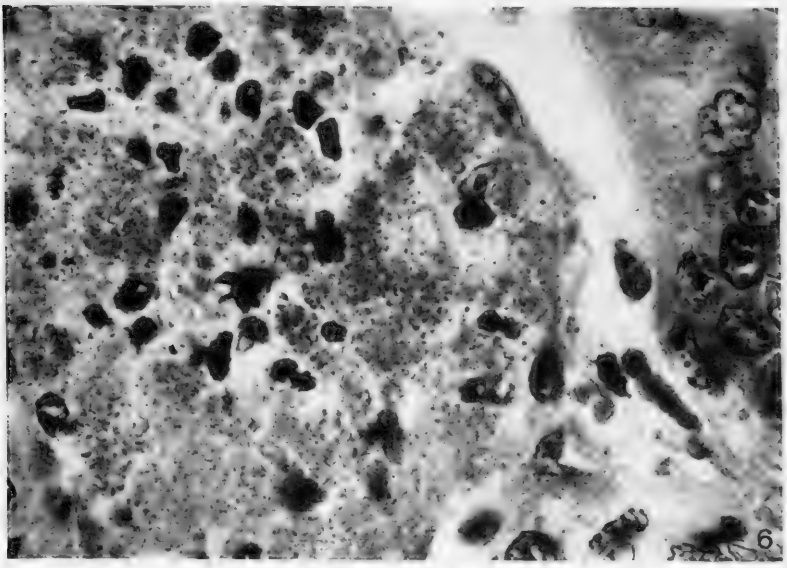


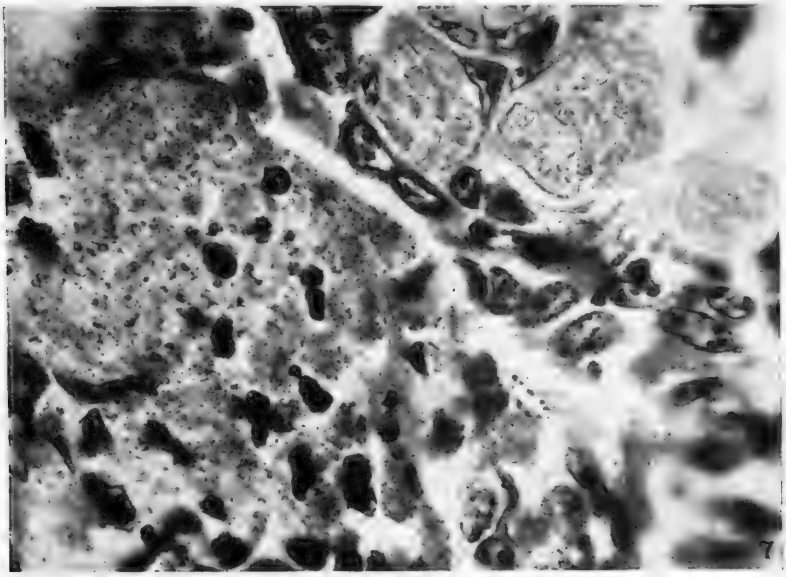
(Smith: *Bacillus actinoides.*)



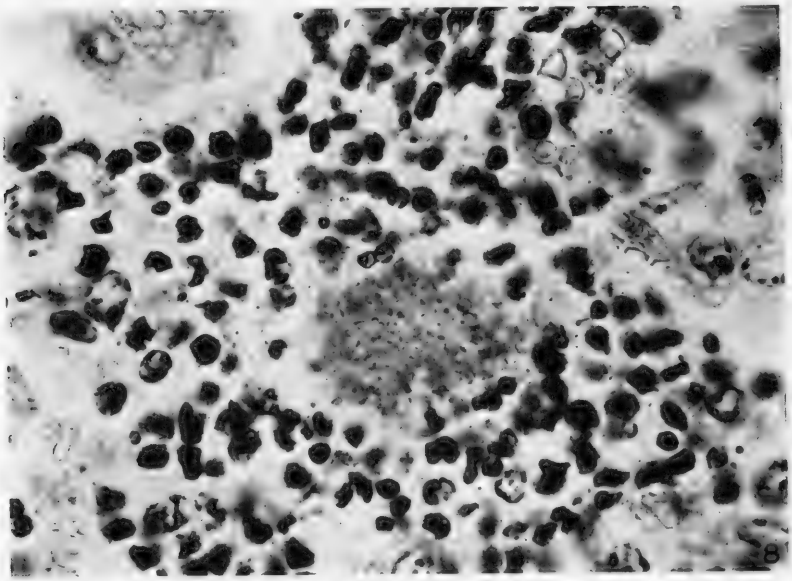


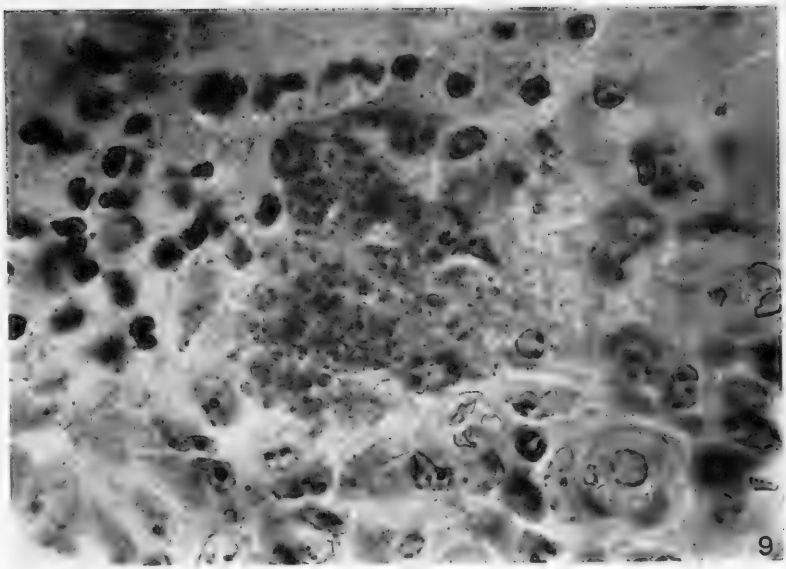
(Smith: *Bacillus actinoides.*)



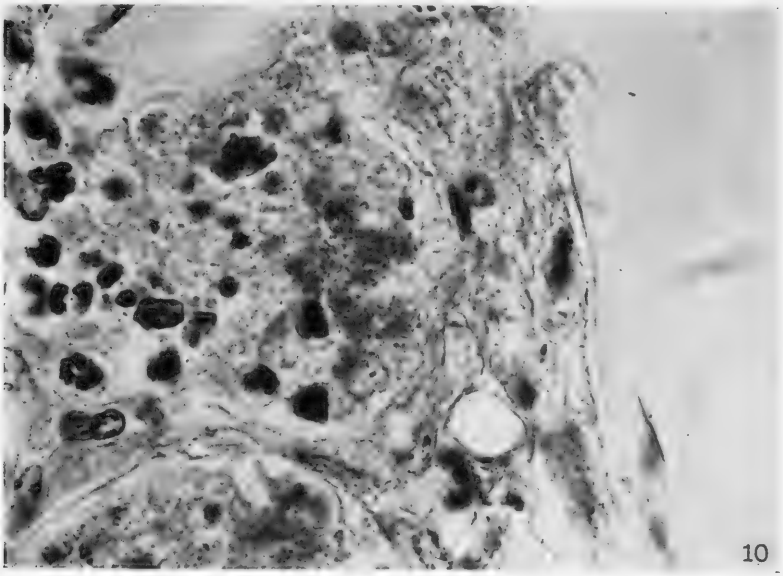


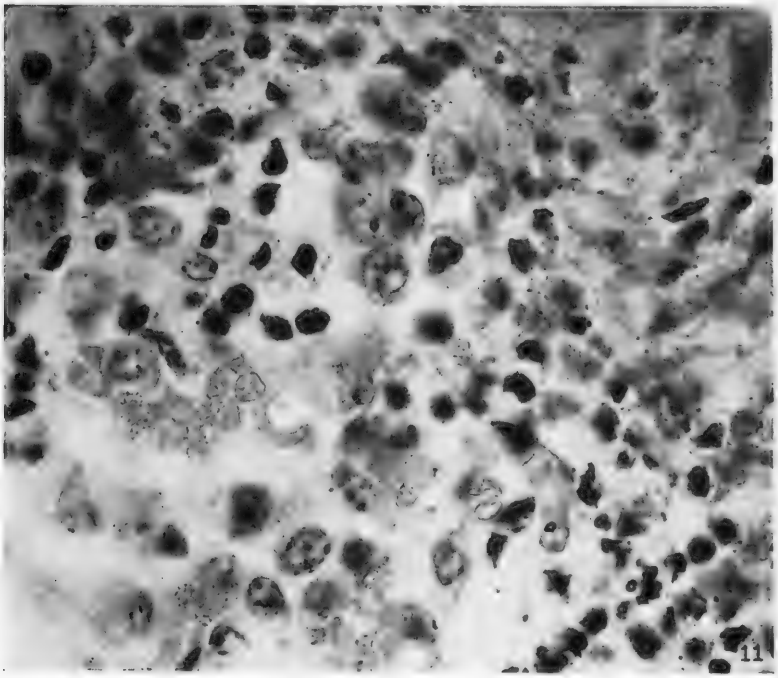
(Smith: *Bacillus actinoides.*)



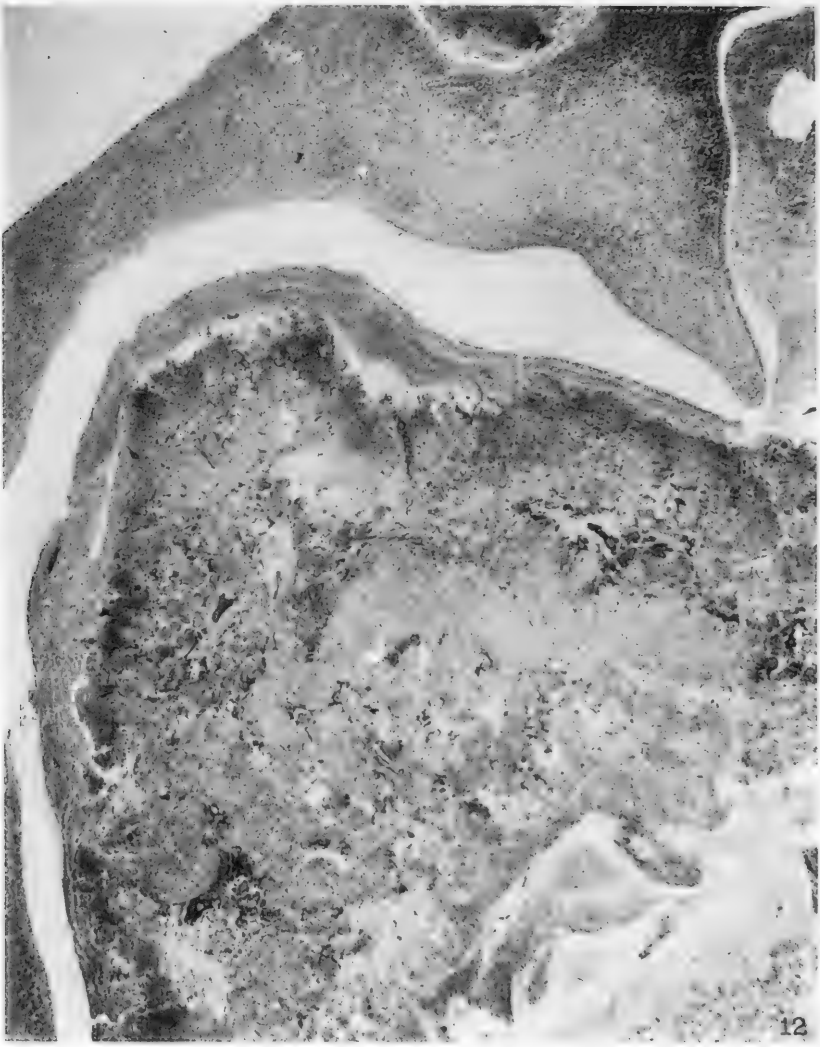


(Smith: *Bacillus actinoides.*)





(Smith: *Bacillus actinoides.*)



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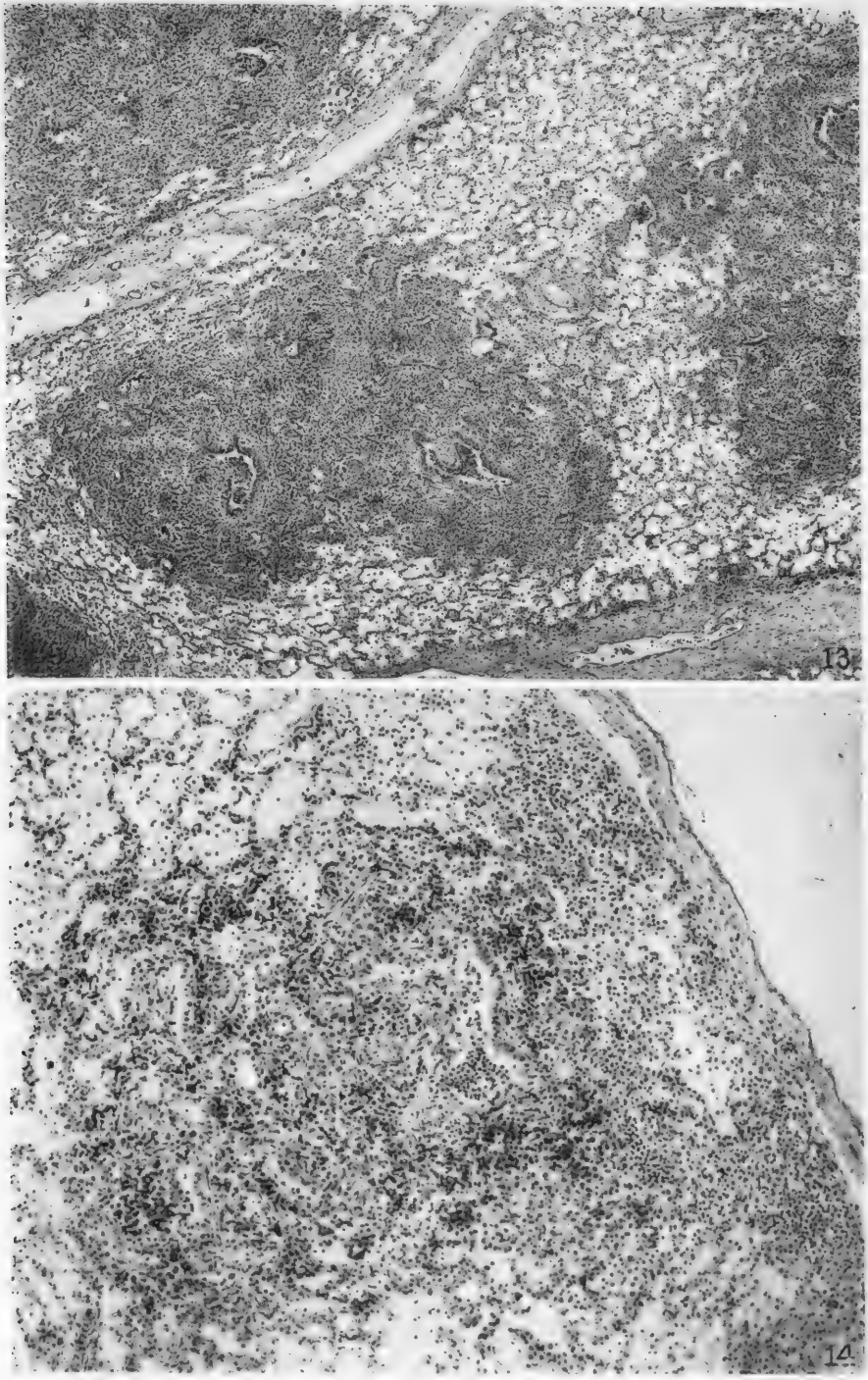

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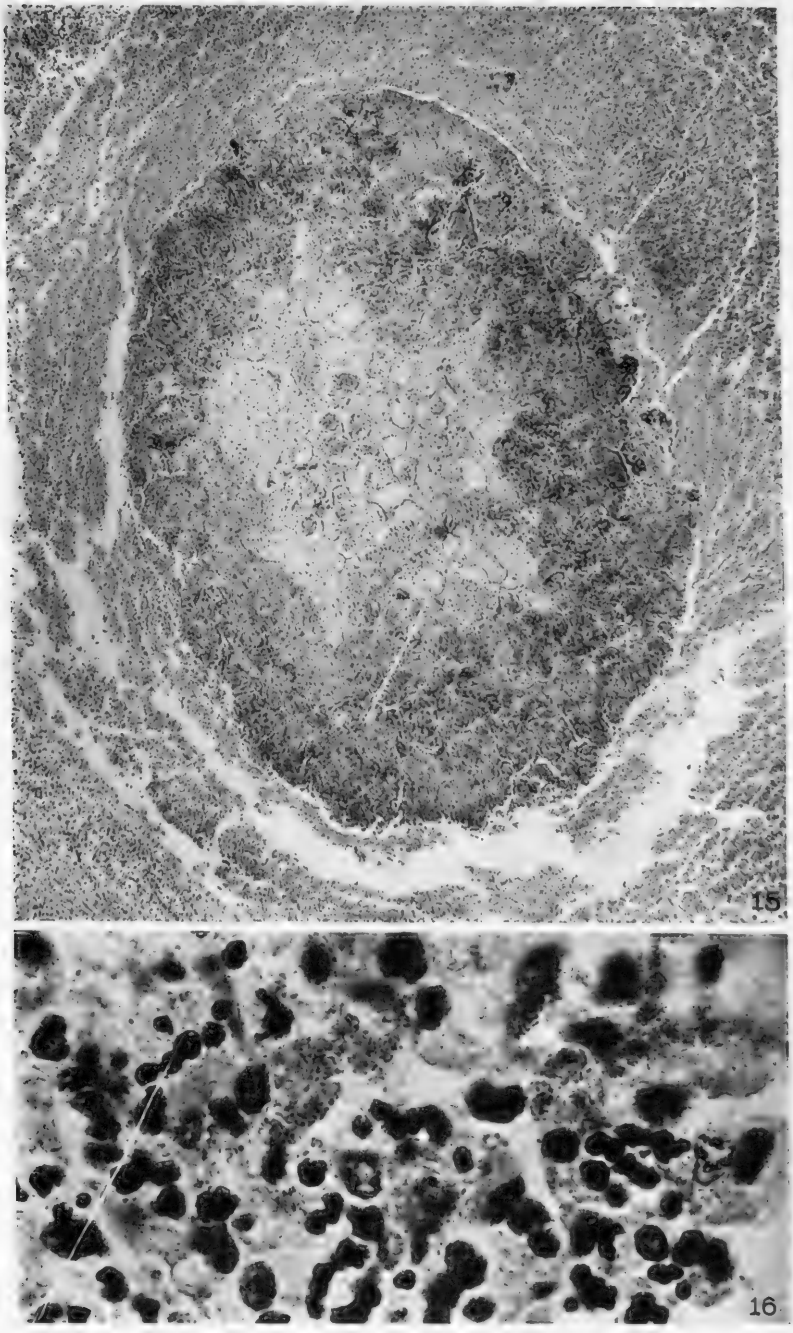

(Smith: *Bacillus actinoides.*)

PRECIPITIN RESPONSE IN THE BLOOD OF RABBITS FOLLOWING SUBARACHNOID INJECTIONS OF HORSE SERUM.

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During the treatment of cases of cerebrospinal meningitis with antimeningococcic serum in a large army hospital[1] a peculiar reaction was repeatedly observed. This reaction appeared in patients who, after having received several intraspinal injections of serum, were given serum intravenously. While the injection was being made into the blood stream, or immediately afterward, some of the following signs and symptoms were frequently noted: flushing, sudden feeling of warmth, and restlessness, followed by pallor, dyspnea, cyanosis, vomiting, and prostration. Hypodermatic injections of epinephrine and atropin brought relief. In the press of work there was no opportunity for detailed blood pressure and temperature studies. These manifestations, apparently anaphylactic, occurred only when several intraspinal treatments were given before initial intravenous serum therapy, and had no relation to the time of the last intraspinal injection. They were not noted when combined intraspinal and intravenous therapy was applied from the outset. Similar observations are described by Stone and Truitt[2] in a report of a large series of cases of cerebrospinal meningitis, and Haden[3] mentions intolerance to antimeningococcic serum under like conditions.

With this experience in mind, horse serum was injected into rabbits intraspinally and the resulting precipitin formation in the blood

[1] Hospital of the American Embarkation Center, Le Mans, France.

[2] Stone, W. J., and Truitt, R. C. P., *Arch. Int. Med.*, 1919, xxiii, 282.

[3] Haden, R. L., *Arch. Int. Med.*, 1919, xxiv, 514.

was compared with that induced by similar intravenous injections. In a few instances anaphylactins were studied.

The formation of specific precipitins, in the blood, to foreign proteins was first recorded by Kraus[4] in 1897. Since then, numerous methods have been described for inducing the formation of these bodies in high titer.[5, 6,.7] Protein is injected into susceptible animals, either *via* the blood stream or peritoneum, the methods differing chiefly in dose and interval.

Weed[8] has clearly shown the pathway by which substances injected into the subarachnoid space readily find access to the blood stream. On the other hand, most substances, including circulating toxins and antibodies (agglutinins to *B. typhosus*, tetanus toxin, and hemolytic amboceptors)[9, 10, 11] are normally excluded from the spinal fluid by means of the meningeal-choroid complex which acts as an effective barrier to them. Artificial damage to the meninges, however, by such agents as normal horse serum and even normal salt solution, makes them permeable to immune bodies. Flexner and Amoss[12] thus recovered neutralizing principles to the virus of anterior poliomyelitis from the spinal fluid, after intravenous injection into monkeys. More recently, Amoss and Eberson[13] by similar methods detected meningococcic agglutinins in the cerebrospinal fluid after their introduction into the blood stream.

EXPERIMENTAL.

Normal horse serum, without preservative, was used throughout the experiments. It was injected into the subarachnoid space of rabbits by introducing a No. 24 Luer needle attached to a glass syringe, through a sterile field just below the occipital ridge in the midline. The needle was carried forward and slightly downward until it punctured the occipito-atlantoid ligament. A yield of from 0.5 to 1 cc. of cerebrospinal fluid was thus readily obtained. The needle was left in place, the syringe disconnected, and a second syringe

[4] Kraus, R., *Wien. klin. Woch.*, 1897, x, 736.

[5] Kolmer, J. A., A practical text-book of infection, immunity and specific therapy, Philadelphia and London, 2nd edition, 1917.

[6] Zinsser, H., Infection and resistance, New York, 2nd edition, 1918.

[7] Hektoen, L., *J. Infect. Dis.*, 1917, xxi, 279.

[8] Weed, L. H., *Anat. Rec.*, 1917, xii, 461.

[9] Mott, F. W., *Lancet*, 1910, ii, 1.

[10] Ransom, F., *Z. physiol. Chem.*, 1900, xxxi, 282.

[11] Cushing, H., *J. Med. Research*, 1914–15, xxxi, 1.

[12] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 499.

[13] Amoss, H. L., and Eberson, F., *J. Exp. Med.*, 1919, xxix, 597.

with the correct amount of serum was attached. After slightly withdrawing the plunger to prevent any chance puncture of a vessel the serum was slowly injected.

Precipitin tests were made by mixing 0.3 cc. of rabbit serum with 0.3 cc. of normal horse serum (for unchanged horse serum, equal parts of an anti-horse rabbit serum with dilutions of the serum or cerebrospinal fluid to be tested). After incubation at 37°C. for 1 hour in a water bath the tubes were placed in the ice chest over night and readings were made the following morning. As controls, normal rabbit serum and normal sheep serum were used. In all instances as controls for rabbits receiving subarachnoid injections other rabbits were injected with identical amounts of the same serum intravenously, and the bleedings and precipitin tests of each were done at the same time.

The following protocols are selected as typical of the results obtained. Text-figs. 1 to 3 are graphic charts of precipitin tests in each experiment.

Experiment 1.—Rabbit 1; weight 1,860 gm. Feb. 6, 1920. Subarachnoid puncture. 0.5 cc. of cerebrospinal fluid removed. 0.5 cc. of normal horse serum injected. No reaction.

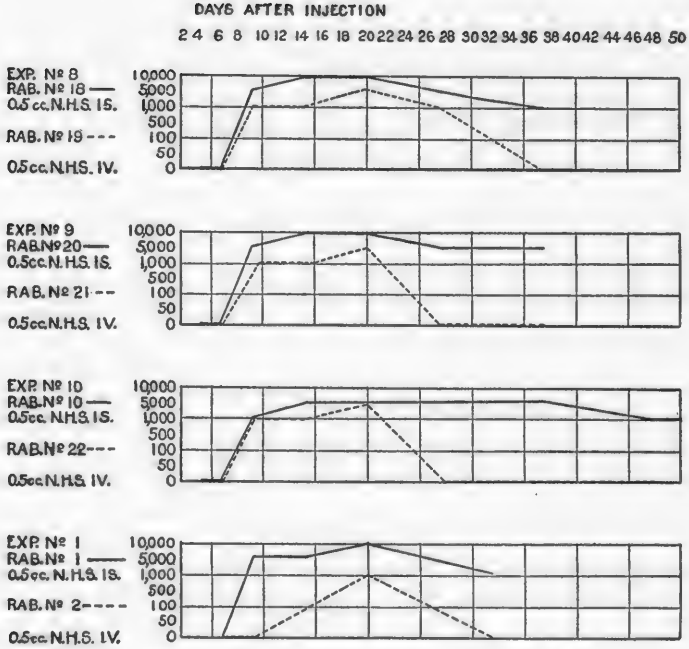
Rabbit 2; weight 1,650 gm. Feb. 6, 1920. 0.5 cc. of normal horse serum injected intravenously.

Precipitin Tests with Sera 1 and 2 against Dilutions of Normal Horse Serum.

Date.	1:10		1:100		1:500		1:1,000		1:5,000		1:10,000	
	Serum 1.	Serum 2.	Serum 1.	Serum 2.	Serum 1.	Serum 2.	Serum 1.	Serum 2.	Serum 1.	Serum 2.	Serum 1	Serum 2.
1920												
Feb. 12	0	0	0	0	0	0	0	0	0	0	0	0
" 15	±	±	++	±	++	0	++	0	+	0	±	0
" 20	0	+	+	+	++	±	++	0	+	0	0	0
" 26	++	0	++	+	++	++	++	+	++	±	+	0
Mar. 9	±	0	+	0	++	0	++	0	−	0	0	0

Controls: normal rabbit serum and normal sheep serum against dilutions of normal horse serum, negative.

In the tables +++ indicates very heavy precipitate; ++, heavy precipitate; +, distinct precipitate; ±, questionable precipitate; 0, no precipitate; −, no test.


DAYS AFTER INJECTION
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
EXP. Nº 8
RAB. Nº 18 —
0.5 cc. N.H.S. IS.
RAB. Nº 19 - - -
0.5 cc. N.H.S. IV.
10,000
5,000
1,000
500
100
50
0
EXP. Nº 9
RAB. Nº 20 —
0.5 cc. N.H.S. IS.
RAB. Nº 21 - -
0.5 cc. N.H.S. IV.
EXP. Nº 10
RAB. Nº 10 —
0.5 cc. N.H.S. IS.
RAB. Nº 22 - - -
0.5 cc. N.H.S. IV.
EXP. Nº 1
RAB. Nº 1 —
0.5 cc. N.H.S. IS.
RAB. Nº 2 - - - -
0.5 cc. N.H.S. IV.

In the text-figures N.H.S. indicates normal horse serum; I S., intraspinally; I V., intravenously.

TEXT-FIG. 1. Precipitin tests after one intraspinal or intravenous injection of normal horse serum.

Experiment 2.—Rabbit 3; weight 2,100 gm. Mar. 30, 1920. Subarachnoid puncture. 0.5 cc. of cerebrospinal fluid removed. 0.5 cc. of normal horse serum injected. No reaction.

Rabbit 4; weight 2,100 gm. Mar. 30, 1920. 0.5 cc. of normal horse serum injected intravenously.

Precipitin Tests with Sera 3 and 4 against Dilutions of Normal Horse Serum.

Date.	1:10		1:100		1:500		1:1,000		1:5,000		1:10,000	
	Serum 3.	Serum 4.	Serum 3.	Serum 4.	Serum 3.	Serum 4.	Serum 3.	Serum 4.	Serum 3.	Serum 4.	Serum 3.	Serum 4.
1920												
Apr. 5	0	0	0	0	0	0	0	0	0	0	0	0
" 8	±	0	++	+	++	+	++	0	+	0	+	0
" 13	+	0	++	+	++	+	++	+	+	+	+	0
" 19	0	0	+	+	++	++	++	+	+	+	+	0
" 26	+	0	+	0	+	0	+	0	±	0	0	0
May 3	0	0	+	0	+	0	±	0	0	0	0	0

Controls: normal rabbit serum and normal sheep serum against dilutions of normal horse serum, negative.

Experiment 3.—Rabbit 5; weight 1,920 gm. Mar. 30, 1920. Subarachnoid puncture. 0.5 cc. of cerebrospinal fluid removed. 0.5 cc. of normal horse serum injected. No reaction.

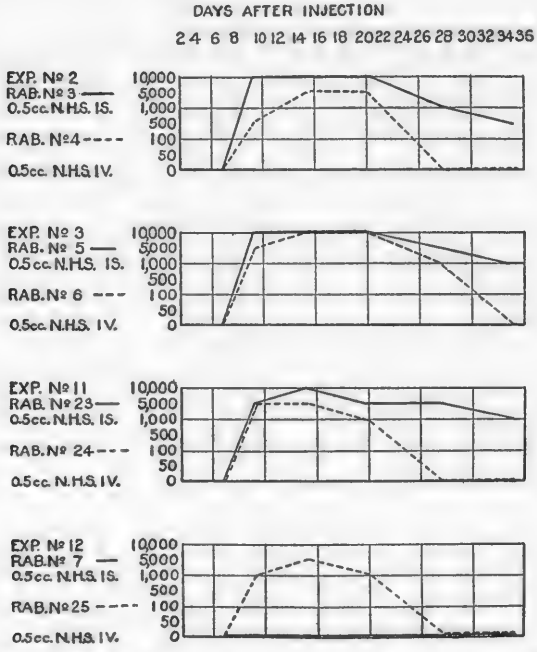
Rabbit 6; weight 2,000 gm. Mar. 30, 1920. 0.5 cc. of normal horse serum injected intravenously.

Precipitin Tests with Sera 5 and 6 against Dilutions of Normal Horse Serum.

Date.	1:10		1:100		1:500		1:1,000		1:5,000		1:10,000	
	Serum 5.	Serum 6.	Serum 5.	Serum 6.	Serum 5.	Serum 6.	Serum 5.	Serum 6.	Serum 5.	Serum 6.	Serum 5.	Serum 6.
1920												
Apr. 5	0	0	±	0	+	0	±	0	0	0	0	0
" 8	++	+	++	+	+++	+	++	+	++	+	+	±
" 13	+	0	++	++	++	++	++	++	++	+	+	+
" 19	0	±	++	++	++	++	++	++	++	+	+	+
" 26	±	0	++	+	++	+	++	+	+	±	0	0
May 3	++	0	++	0	++	0	+	0	±	0	0	0

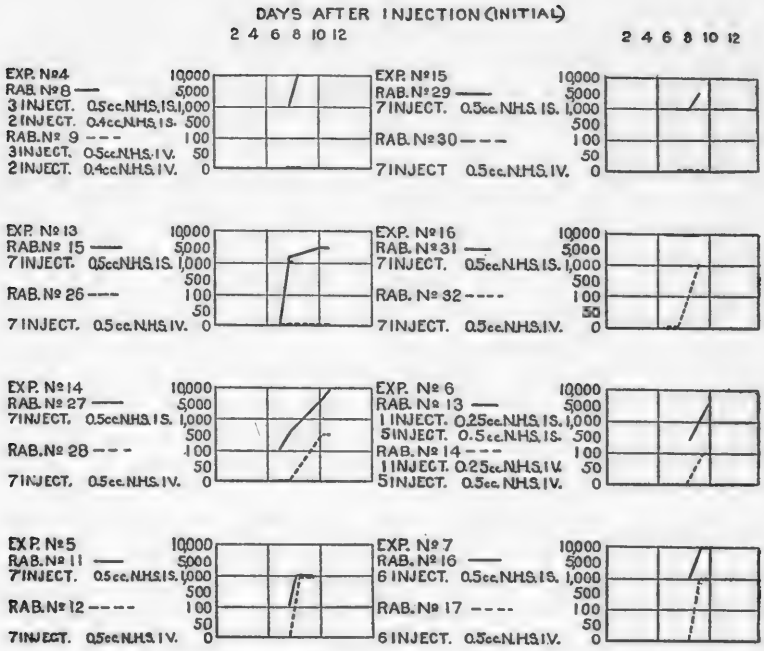
Controls: normal rabbit serum and normal sheep serum against dilutions of normal horse serum, negative.

In these experiments, in which single injections of normal horse serum were made intraspinally and intravenously, precipitins appeared in the blood stream in both instances on approximately the same days. Rabbits receiving serum intraspinally, however, showed precipitins in higher titers, which persisted after the disappearance of precipitins in rabbits treated intravenously. In the one instance



TEXT-FIG. 2. Precipitin tests after one intraspinal or intravenous injection of normal horse serum.

that was followed precipitins after intraspinal injections were present in the blood in a titer of 1:1,000 on the 56th day after injection, whereas in the control rabbit, inoculated intravenously, they had disappeared by the 27th day. The single exception appeared in Rabbit 7, in which no precipitins were found after subarachnoid injections, whereas the control showed a normal precipitin curve



TEXT-FIG. 3. Precipitin tests after repeated intraspinal or intravenous injections of normal horse serum.

(Text-fig. 2). It is common to find rabbits which fail to give any precipitin response whatever to protein injections.[5]

In the next series of experiments repeated intraspinal injections of normal horse serum were given to rabbits; as controls others were so treated intravenously. The following protocols are typical.

Experiment 4.—Rabbit 8; weight 2,500 gm. Jan. 14, 1920. Subarachnoid puncture. 0.7 cc. of cerebrospinal fluid removed. 0.5 cc. of normal horse serum injected. Jan. 15. Subarachnoid puncture. 0.5 cc. of cerebrospinal fluid removed. 0.5 cc. of normal horse serum injected. Jan. 17. Subarachnoid puncture. 0.5 cc. of cerebrospinal fluid removed. 0.5 cc. of normal horse serum injected. Jan. 20. Subarachnoid puncture. 0.5 cc. of cerebrospinal fluid removed. 0.4 cc. of normal horse serum injected. Jan. 22. Subarachnoid puncture. 0.2 cc. of cerebrospinal fluid removed. 0.4 cc. of normal horse serum injected.

Rabbit 9; weight 2,350 gm. Jan. 14, 1920. 0.5 cc. of normal horse serum injected intravenously. Jan. 15. 0.5 cc. of normal horse serum injected intravenously. Jan. 17. 0.5 cc. of normal horse serum injected intravenously. Jan. 20. 0.4 cc. of normal horse serum injected intravenously. Jan. 22. 0.4 cc. of normal horse serum injected intravenously.

Precipitin Tests with Sera 8 and 9 against Dilutions of Normal Horse Serum.

Date.	1:10		1:100		1:500		1:1,000		1:5,000		1:10,000	
	Serum 8.	Serum 9.	Serum 8.	Serum 9.	Serum 8.	Serum 9.	Serum 8.	Serum 9.	Serum 8.	Serum 9.	Serum 8.	Serum 9.
1920												
Jan. 21	+	0	+++	0	++	0	+	0	±	0	0	0
" 22	+++	0	+++	0	−	0	+++	0	++	0	+	0

Controls: normal rabbit serum and normal sheep serum against dilutions of normal horse serum, negative.

Precipitin Tests with Dilutions of Sera 8 and 9 against Serum 10, an Anti-Horse Rabbit Serum, in Order to Detect Unchanged Horse Serum (Precipitinogen) in the Blood.

Date.	1:10		1:100		1:500		1:1,000	
	Serum 8.	Serum 9.	Serum 8.	Serum 9.	Serum 8.	Serum 9.	Serum 8.	Serum 9.
1920								
Jan. 15	+	−	+	−	0	−	−	−
" 17	++	−	+	−	±	−	0	−
" 21	±	++	0	+	0	0	0	−

Precipitin Tests with Dilutions of Cerebrospinal Fluid 8 against Serum 10, an Anti-Horse Rabbit Serum, in Order to Detect Unchanged Horse Serum (Precipitinogen) in the Cerebrospinal Fluid.

Date.	1:10	1:100	1:500	1:1,000
1920				
Jan. 15	++	+	0	−
" 17	++	+	+	0

Experiment 5.—Rabbit 11; weight 1,780 gm. Mar. 2, 3, 5, 6, 8, 9, and 10, 1920. On each of these days a subarachnoid puncture was made, 0.5 cc. of cerebrospinal fluid was withdrawn, and 0.5 cc. of normal horse serum injected.

Rabbit 12; weight 1,900 gm. Mar. 2, 3, 5, 6, 8, 9, and 10, 1920. On each of these days 0.5 cc. of normal horse serum was injected intravenously.

Precipitin Tests with Sera 11 and 12 against Dilutions of Normal Horse Serum.

Date.	1:10		1:100		1:500		1:1,000		1:5,000		1:10,000	
	Serum 11.	Serum 12.	Serum 11.	Serum 12.	Serum 11.	Serum 12.	Serum 11.	Serum 12.	Serum 11.	Serum 12.	Serum 11.	Serum 12.
1920												
Mar. 9	±	0	+	0	±	0	0	0	0	0	0	0
" 10	+	0	++	+	++	+	+	+	0	0	0	0
" 11	++	+	++	++	++	+	+	+	0	0	0	0

Controls: normal rabbit serum and normal sheep serum against dilutions of normal horse serum, negative.

Precipitin Tests with Dilutions of Sera 11 and 12 against Serum 1, an Anti-Horse Rabbit Serum, in Order to Detect Unchanged Horse Serum (Precipitinogen) in the Blood.

Date.	1:10		1:100		1:500		1:1,000	
	Serum 11.	Serum 12.	Serum 11.	Serum 12.	Serum 11.	Serum 12.	Serum 11.	Serum 12.
1920								
Mar. 8	±	+++	0	+	0	0	0	0
" 9	+	+++	±	++	0	±	0	0
" 10	+	++	0	0	0	0	0	0

Precipitin Tests with Dilutions of Cerebrospinal Fluid 11 against Serum 1, an Anti-Horse Rabbit Serum, in Order to Detect Unchanged Horse Serum (Precipitinogen) in the Cerebrospinal Fluid.

Date.	1:10	1:100	1:500	1:1,000
1920				
Mar. 8	0	0	0	0
" 9	++	+	±	0
" 10	+	+	0	−

Experiment 6.—Rabbit 13; weight 1,960 gm. June 15, 1920. Subarachnoid puncture. 0.5 cc. of cerebrospinal fluid was removed. 0.25 cc. of normal horse serum injected. June 17, 18, 19, 21, and 23. On each of these days a subarachnoid puncture was made, 0.5 cc. of cerebrospinal fluid was removed, and 0.5 cc. of normal horse serum injected.

Rabbit 14; weight 2,100 gm. June 15, 1920. 0.25 cc. of normal horse serum was injected intravenously. June 17, 18, 19, 21, and 23. On each of these days 0.5 cc. of normal horse serum was injected intravenously.

Precipitin Tests with Sera 13 and 14 against Dilutions of Normal Horse Serum.

Date.	1:10		1:100		1:500		1:1,000		1:5,000		1:10,000	
	Serum 13.	Serum 14.	Serum 13.	Serum 14.	Serum 13.	Serum 14.	Serum 13.	Serum 14.	Serum 13.	Serum 14.	Serum 13.	Serum 14.
1920												
June 23	±	0	+	0	+	0	0	0	0	0	0	0
" 24	+	±	++	+	++	0	+	0	0	0	0	0
" 25	++	+	++	+	++	±	+	0	+	0	0	0

Controls: normal rabbit serum and normal sheep serum against dilutions of normal horse serum, negative.

Precipitin Tests with Dilutions of Sera 13 and 14 against Serum 15, an Anti-Horse Rabbit Serum, in Order to Detect Unchanged Horse Serum (Precipitinogen) in the Blood.

Date.	1:10		1:100		1:500		1:1,000	
	Serum 13.	Serum 14.	Serum 13.	Serum 14.	Serum 13.	Serum 14.	Serum 13.	Serum 14.
1920								
June 21	+	+	0	+	0	0	0	0
" 22	+	++	0	+	0	0	0	0
" 23	±	++	0	+	0	0	−	0

Precipitin Tests with Dilutions of Cerebrospinal Fluid 13 against Serum 15, an Anti-Horse Rabbit Serum, in Order to Detect Unchanged Horse Serum (Precipitinogen) in the Cerebrospinal Fluid.

Date.	1:10	1:100	1:500	1:1,000
1920				
June 21	+	0	0	0
" 22	+	0	0	0
" 23	±	0	0	−

June 25, 1920, 2.30 p.m. Guinea Pig 1, 1 cc. of Rabbit Serum 13[14] intraperitoneally. Guinea Pig 2, 2.5 cc. of Rabbit Serum 13 intraperitoneally. Guinea Pig 3, 1 cc. of Rabbit Serum 14[14] intraperitoneally. Guinea Pig 4, 2.5 cc. of Rabbit Serum 14 intraperitoneally. June 26, 4 p.m. Guinea Pig 1, 0.5 cc. of normal horse serum intracardially; mild anaphylactic symptoms. Guinea Pig 2, 0.5 cc. of normal horse serum intracardially; anaphylactic death in 3 minutes. Guinea Pig 3, 0.5 cc. of normal horse serum intracardially; no anaphylactic symptoms. Guinea Pig 4, 0.5 cc. of normal horse serum intracardially; no anaphylactic symptoms.

Experiment 7.—Rabbit 16; weight 2,100 gm. June 15, 17, 18, 19, 21, and 23, 1920. On each of these days a subarachnoid puncture was made, 0.5 cc. of cerebrospinal fluid was withdrawn, and 0.5 cc. of normal horse serum injected.

Rabbit 17; weight 2,040 gm. June 15, 17, 18, 19, 21, and 23, 1920. On each of these days 0.5 cc. of normal horse serum was injected intravenously.

Precipitin Tests with Sera 16 and 17 against Dilutions of Normal Horse Serum.

Date.	1:10		1:100		1:500		1:1,000		1:5,000		1:10,000	
	Serum 16.	Serum 17.	Serum 16.	Serum 17.	Serum 16.	Serum 17.	Serum 16.	Serum 17.	Serum 16.	Serum 17.	Serum 16.	Serum 17.
1920												
June 23	+	0	++	0	++	0	+	0	±	0	0	0
" 24	++	±	++	+	++	+	++	+	+	0	+	0
" 25	++	+	++	++	++	+	++	+	+	0	+	0

Controls: normal rabbit serum and normal sheep serum against dilutions of normal horse serum, negative.

Precipitin Tests with Dilutions of Sera 16 and 17 against Serum 15, an Anti-Horse Rabbit Serum, in Order to Detect Unchanged Horse Serum (Precipitinogen) in the Blood.

Date.	1:10		1:100		1:500		1:1,000	
	Serum 16.	Serum 17.	Serum 16.	Serum 17.	Serum 16.	Serum 17.	Serum 16.	Serum 17.
1920								
June 21	+	++	0	+	0	0	0	0
" 22	±	+	0	±	0	0	0	0
" 23	0	+	0	0	0	0	0	0

[14] These sera given on June 25 were withdrawn just prior to guinea pig inoculations.

Precipitin Tests with Dilutions of Cerebrospinal Fluid 16 against Serum 15, an Anti-Horse Rabbit Serum, in Order to Detect Unchanged Horse Serum (Precipitinogen) in the Cerebrospinal Fluid.

Date.	1:10	1:100	1:500	1:1,000
1920				
June 21	+	0	0	0
" 22	+	0	0	0
" 23	Lost.	Lost.	Lost.	Lost.

June 25, 1920, 2.25 p.m. Guinea Pig 5, 0.1 cc. of Rabbit Serum 16 intraperitoneally. Guinea Pig 6, 1 cc. of Rabbit Serum 16 intraperitoneally. Guinea Pig 7, 2 cc. of Rabbit Serum 16 intraperitoneally. Guinea Pig 8, 0.1 cc. of Rabbit Serum 17 intraperitoneally. Guinea Pig 9, 1 cc. of Rabbit Serum 17 intraperitoneally. Guinea Pig 10, 2 cc. of Rabbit Serum 17 intraperitoneally. June 26, 4 p.m. Guinea Pig 5, 0.5 cc. of normal horse serum intracardially; no anaphylactic symptoms. Guinea Pig 6, 0.5 cc. of normal horse serum intracardially; severe anaphylactic symptoms. Guinea Pig 7, 0.5 cc. of normal horse serum intracardially; died in 4 minutes. Guinea Pig 8, 0.5 cc. of normal horse serum intracardially; no anaphylactic symptoms. Guinea Pig 9, 0.5 cc. of normal horse serum intracardially; no anaphylactic symptoms. Guinea Pig 10, 0.5 cc. of normal horse serum intracardially; mild anaphylactic symptoms. Guinea Pig 11 (control; injected May 10 with 1 cc. of normal horse serum intraperitoneally), 0.1 cc. of normal horse serum intracardially; died in 3 minutes.

Text-figs. 1 to 3 represent graphically the precipitin response in all the rabbits. Precipitin tests for unchanged horse serum in the blood and spinal fluid, as well as the strength of all the reactions, are omitted to avoid confusion.

DISCUSSION.

The marked discrepancy between precipitin formation in the blood of rabbits following intraspinal and intravenous injections of the precipitinogen, coincides with differences in clinical manifestations after administration of horse serum by these channels. The explanation of the reactions is not clear. Two alternatives are considered: purely mechanical factors whereby horse serum in the cerebrospinal fluid gains access to the blood more slowly than when injected directly into the veins; and a specific influence upon precipitin formation by nervous tissue cells.

In the first instance, a gradual introduction of the precipitinogen into the blood may conceivably have a different stimulating effect upon precipitin formation than when the entire dose is injected intravenously. This may be especially true when serum is repeatedly given, each subsequent intravenous dose tending to combine and thus to use up precipitins already formed. That horse serum injected into the subarachnoid space is detected there 24 and 48 hours after injection may be due to leakage from the blood in spite of the clear character of the cerebrospinal fluid after frequent punctures. Flexner and Amoss[15] have pointed out that meningeal injury capable of causing permeability is not always sufficient to cause morphological changes in the fluid. Mention may be made of experiments by Jacob,[16] who demonstrated iodine and other substances in cells of the central nervous tissues several days after intraspinal administration. In the studies reported here no parallel experiments with intraperitoneal and subcutaneous injections were made. However, it is generally advocated that intravenous administration of the precipitinogen is more effective in precipitin production than the routes mentioned above.

There is little evidence to show that the central nervous system exerts a specific influence on precipitin production and anaphylactic reaction. It is noteworthy, however, that the intracerebral injection of horse serum in sensitized guinea pigs is one of the most rapid and effective methods of producing anaphylactic shock, as pointed out by Besredka and Steinhardt[17] and confirmed by Rosenau and Anderson.[18]

An interesting phase of these experiments is the small amounts of horse serum required to produce potent precipitin formation in contrast to the large doses usually advocated. This experience has recently been confirmed by Mackenzie.[19]

Contrary to expectation, clear cerebrospinal fluid was obtained after repeated intraspinal injections of horse serum which contained no preservative.

[15] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, xxv, 525.

[16] Jacob, P., *Deutsch. med. Woch.*, 1900, xxvi, 46.

[17] Besredka, A., and Steinhardt, E., *Ann. Inst. Pasteur*, 1907, xxi, 117.

[18] Rosenau, M. J., and Anderson, J. F., *Bull. Hyg. Lab., U. S. P. H., No. 45*, 1908.

[19] Mackenzie, G. M., personal communication.

Unfortunately, no cases of meningitis appeared when this subject could be studied clinically. Therefore, whether the clinical reactions noted above are due to a mechanism similar to that in these experiments remains to be proved.

SUMMARY.

1. Rabbits which have received a single dose of normal horse serum in the subarachnoid space produce precipitins in the blood in greater abundance, of higher titer, and persisting longer than those in control rabbits which have received a similar injection intravenously.

2. Repeated subarachnoid injections of normal horse serum in rabbits induce precipitins in the blood early. These may appear in high titer as soon as 1 week after the initial injection, whereas in rabbits similarly treated intravenously no precipitins are found at this time. They may appear a few days afterward and reach a high titer.

3. No anaphylactic manifestations occurred in rabbits treated repeatedly with subarachnoid injections of normal horse serum when the precipitin content of the blood was high.

4. Anaphylactins, as determined by passive transfer of anaphylaxis, were demonstrated in sera with high precipitin content.

5. These experiments may explain clinical evidences of anaphylaxis, observed when an initial intravenous injection of horse serum followed a series of intraspinal injections of such serum.

GRANULES IN THE CELLS OF CHICK EMBRYOS PRODUCED BY EGG ALBUMIN IN THE MEDIUM OF TISSUE CULTURES.

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PLATE 51.

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It has been shown that connective tissue cells of the chick embryo become greatly altered when placed in an abnormal environment (M. R. Lewis, 1918; W. H. Lewis, 1919; M. R. Lewis, 1920; Prigosen, 1921). These alterations in appearance can be compared with certain changes which take place in pathological conditions of adult tissue such as fatty degeneration, necrosis, autolysis, phagocytosis, etc.

The present paper is a report of the results obtained when white of egg is introduced into the medium of a tissue culture of connective tissue. This substance not only has a decidedly toxic influence upon the growth of the cells, but it also produces a marked change in the appearance of the cells comprising the growth, owing to an accumulation of large, somewhat refractive granules within the cytoplasm of these cells. These granules take the eosin stain when methylene blue and eosin are used. In this regard the connective tissue cells resemble the epithelium of renal tubules filled with colloid or hyaline droplets described by MacCallum (1916).[1] On the other hand, when the stain used is iron-hematoxylin, these cells closely resemble certain cells undergoing active secretion described by Hoven (1910), Mislawsky (1913), and Saguchi (1920).

It has been shown that explants of connective tissue from the chick embryo grow in pure egg albumin (Swezy, 1915). These cultures do not live so long or attain so extensive a growth as cultures explanted in Locke-Lewis solution. Cells from the subcutaneous tissue

[1] MacCallum (1916), p. 91.

of the chick embryo, explanted into egg albumin, become filled with large round granules of different sizes (Figs. 1 and 2). It has not been possible so far to demonstrate the exact chemical nature of these granules; therefore, in order to differentiate them from other granules observed in the cells, they will be called al. granules throughout this paper. This term is not meant to signify that these granules are or are not albumin, but only to specify that they are due to the presence of albumin in the environment of the cells.

Chick Embryo Connective Tissue in Egg Albumin.

An egg was opened under sterile conditions and a small quantity of the white removed to a sterile dish. This was then cut many times with sharp curved scissors in order to obtain a solution of egg albumin. A piece of connective tissue was removed from a chick embryo of 7 to 10 days incubation and placed on the solution of egg white where it was cut up into small pieces. Each piece, with a small quantity of egg albumin, was placed on a clean cover-glass and inverted over a vaseline ring on a depression slide. After 16 to 20 hours connective tissue cells had grown out into the medium in the same manner as in normal cultures, though by no means in such great numbers or to so great an extent. All these cells contained a number of rather large granules, usually round in outline. The granules were composed of a substance which differed in appearance from the cytoplasm. They seemed to be denser, more opaque, and possessed an index of refraction different from that of the cytoplasm. In fixed preparations they stained a much deeper tone than the cytoplasm, becoming black and gray with hematoxylin but never so pale as the cytoplasm. There was great variation in the size of the granules in a given cell, as well as in those in different cells. They were in most cases much larger than either the mitochondrial bodies or the neutral red granules.

Shipley (1919)[2] described somewhat similar granules in plasma cultures of chick embryos. He stated, however,[3] that the degeneration vacuoles described by Lewis and Lewis were identical with these

[2] Shipley (1919), p. 288.
[3] Shipley (1919), p. 289.

plasma granules, and that the differences in the two bodies were due to differences in the media. This is not true in regard to the al. granules characteristic of the albumin cultures, for the two bodies, *i.e.* the al. granule and the degeneration vacuole (W. H. Lewis, 1919), may exist side by side in degenerating cultures (Figs. 3 and 5). In such cultures the al. granules were markedly different from the degeneration vacuoles in appearance and were easily distinguishable from them, not only in the living cell but also in fixed material.

Plato (1900) injected dried and powdered white of egg into the abdominal cavity of a guinea pig. Shortly afterwards leucocytes were obtained from this region and stained with neutral red. They were found to contain large, irregularly shaped, orange-red clumps, which Plato supposed were ingested particles of white of egg taken in as foreign bodies. The structures obtained in this manner do not resemble to any great extent those described above as al. granules.

After 24 to 48 hours a difference could sometimes be detected in the appearance of the individual al. granules in a given cell. Some appeared to be of a different consistency, but whether this could be expressed as more fluid is doubtful. In the stained preparations certain of the granules were much darker than others (Fig. 2) and the changed, possibly more fluid, granules of the living cell probably correspond to the more lightly stained ones in fixed cultures.

The al. granules differed from certain other types of granules in living cells, such as pigment granules, neutral red granules, and certain secretion granules, in that they were less frequently found collected around the centrosphere (Figs. 2 and 5) than in other regions of the cytoplasm. The processes of the cells were always free from al. granules. Frequently there was an extensive ectoplasm which the granules did not enter, but occasionally this was reduced to a narrow ectosarc on one or both sides of the nucleus. These granules obscured the other granules in the living cell, except at the periphery, where the mitochondria could be seen extending out into processes of the cell. Spindle-shaped cells (Fig. 1) were more numerous than were the flat cells (Fig. 2). These elongated cells contained granules in the neighborhood of the nucleus but there were none in the ends of the cells. The cells divided by mitosis, even when full of granules (Fig. 4). This is another means of differentiating these granules from

the degeneration vacuoles, as cells containing vacuoles rarely undergo mitotic division.

As a rule, all the connective tissue cells of the growth contained some al. granules. Certain other kinds of cells in the same culture, however, did not have these granules. While the number of these bodies in a given connective tissue cell increased to some extent with the age of the cultures, most of the cells contained the maximum number at the end of 24 hours.

It was not possible to observe the fate of the granules in cultures grown in egg albumin, because degeneration took place too rapidly. Generally, after 48 hours, degeneration vacuoles began to appear. These signs of degeneration took place first in the cells along the periphery of the growth. In most of the flat cells the cytoplasm was sufficiently spread out so that it could be readily seen that the region of the centrosphere was free from al. granules and that a number of degeneration vacuoles had collected there (Figs. 3 and 5). From the location of the degeneration vacuoles it is evident that they were not formed from al. granules which had become fluid due to digestion of the substance forming them. After 72 hours vacuoles were present between some of the al. granules, as well as in the region of the centrosphere (Fig. 5), and many of the cells were so degenerate that it was impossible to determine whether the vacuoles were around the granules or between them. In some of the degenerate cultures the al. granules were small and fragmented. After the vacuoles appeared the cells rapidly assumed a rounded form so that the details of the phenomenon could not be observed clearly. The vacuoles increased in number, and degeneration took place much more rapidly than in the normal cultures. Few of the cultures in egg albumin survived longer than 3 or 4 days, while most of the control cultures grown in Locke-Lewis solution lived for over 2 weeks.

Whether the albumin was taken from a freshly laid or from an incubated egg made no difference in the appearance of the al. granules, although the hydrogen ion concentration of an incubated egg differs from that of an unincubated egg. The cells of cultures made with albumin from an incubated egg also divided by mitosis (Fig. 9) and produced a small growth around the explant. After a few days, degeneration took place and the cells died about as rapidly as in albumin from the fresh egg.

Cultures in Diluted Egg Albumin.

Much the same result in regard to the accumulation of granules in the cells was produced when the albumin was diluted one-half with Locke-Lewis solution; but when the quantity of albumin was greatly reduced the results became less constant. This may have been partly due to the difficulty of obtaining a uniform solution of the egg albumin. In media containing only a small percentage of egg albumin (1 to 5 per cent) many of the cultures exhibited no accumulation of granules in the cells (Figs. 6 and 7); in others a few of the cells contained many granules, the remaining cells being entirely free from them; while in still other cultures a few small granules were present in every cell. Some cultures in diluted egg albumin remained normal for several days but later a number of the cells exhibited typical al. granules. The smaller the percentage of albumin in the medium, the more nearly normal did the cultures appear in extent of growth and length of life; in none of the percentages of albumin used did the growths become so large or live so long as they did in the Locke-Lewis solution.

Changes Produced in Cultures When the Locke-Lewis Solution Is Replaced by Egg Albumin.

The hanging drop was removed from 24 to 48 hour cultures of chick connective tissue which had been explanted in Locke-Lewis solution and was replaced by egg albumin. This caused immediate changes in the appearance of the normal connective tissue cells (Fig. 11), due not only to the change in the nature of the environment from a neutral or slightly acid fluid to a markedly alkaline jelly, but also to the manipulation. The mitochondria usually assumed the form of short rods and granules instead of filaments, and the cells became somewhat rounded, showing a number of processes. In some instances many of the cells died. Al. granules did not appear for several hours, but after 20 hours these bodies were about as abundant and of the same size as those in cells of cultures explanted directly into egg albumin. It was not observed that the mitochondria stored up albumin and changed into the al. granules. They did, however, become shorter and seemed to decrease

in number as the cells became full of granules. The size of the growth of cultures under these conditions was always larger than that of cultures explanted directly into egg albumin, because the growth had already become extensive in the normal medium before the egg albumin was placed upon it. When these cultures degenerated, vacuoles appeared in the cytoplasm in the usual manner, regardless of the number or size of al. granules in the cells. Some of the cells degenerating under these conditions presented unusual appearances, especially in the region of the centrosphere. In some instances large bodies resembling a certain type of giant centrosphere described by W. H. Lewis (1920) were observed (Fig. 12).

Effect Produced by Vital Dyes.

When a solution of Janus green was placed upon a culture the cells of which contained al. granules, the mitochondria became a bright blue-green, while the al. granules remained unstained. After the cells began to die, as they soon did owing to the toxic effect of the stain, the al. granules sometimes took on the green color. There were fewer mitochondria in cells living in egg albumin than in those in Locke-Lewis solution and the filaments extending out into the processes were shorter. Scattered among the al. granules were a few, short, rod-shaped and granular mitochondria. In the region of the centrosphere the mitochondria were more like those of normal cultures.

Trypan blue dissolved in Locke-Lewis solution, when placed upon the cultures, failed to stain any body in the cell until a number of hours had elapsed; then a few small blue granules appeared but the al. granules remained colorless (Fig. 8).

Neutral red was taken up by the vacuoles and granules in these cells in about the same manner as in the normal cultures. After a few hours the al. granules were sometimes unstained, again they appeared to be pale pink, and in other cases they seemed to be outlined with red.

Fixed and Stained Preparations.

The al. granules were more successfully fixed and stained than were the mitochondria. Methods of fixation which did not preserve the mitochondria frequently afforded good results in as far as the al. granules were concerned. An easy method of fixation by which both types of granules are preserved is to wash off the albumin by means of warm Locke-Lewis solution and drop the cover-slip into Zenker's solution from which acetic acid has been omitted. With iron-hematoxylin the al. granules stained in various shades, from dense black to gray, the result probably depending upon the concentration of the material forming the granule. In appearance the cells bear a striking resemblance to the secretion cells illustrated by Hoven (1910), Saguchi (1920), Mislawsky (1913), and others. All the forms described by these investigators to show stages in the change from mitochondria into secretion granules can be found. This suggests that Scott (1916) probably was correct in his view that the granules present in certain gland cells may be material accumulated in the cytoplasm and not necessarily formed directly from any preexisting structure of the cell, such as the mitochondria.

Cultures of Fish Embryos in Egg Albumin.

A few cultures of embryos of *Fundulus heteroclitus* were made in egg albumin. The cells of these cultures grew much more slowly but almost as extensively in the egg albumin as did those in the fluid media described by Dederer.[4] Instead of all the mesenchyme cells becoming filled with al. granules, as they do in chick cultures, only a few along the edge of the growth showed the granules. This may be due to the fact, as shown by Dederer, that the mesenchyme cells grow along the cover-slip and are in most places covered by a layer of ectoderm cells which separate them from the medium. The ectoderm did not form al. granules to any appreciable extent. The peripheral mesenchyme cells, which contained al. granules (Fig. 10), appeared much the same as did those of the chick embryo.

[4] Dederer, P. H., personal communication.

DISCUSSION AND SUMMARY.

It is difficult to understand what factors may be concerned in the formation of the al. granules. The phenomenon may be concerned with changes in the cell membrane due to an abnormal environment; that is, material which would otherwise be excluded may be permitted to enter the cell, or, on the other hand, certain substances may be prevented from passing out of the cells. Previous investigators have shown that mesenchyme cells sometimes engulf certain foreign bodies, and it is possible that the solution of white of egg is ingested in the same manner. When a solution of peptone was placed on the cells instead of egg white, the phenomenon did not occur (Fig. 13); the cell remained normal and degenerated in the usual manner (Fig. 14). This would seem to indicate that the al. granules are not formed from peptone. Regardless of the factors involved, it is evident that egg albumin in the medium of tissue cultures of chick embryos causes the formation of numerous large granules in the cytoplasm of the connective tissue cells. This phenomenon is associated with unfavorable conditions for the life of the cells and results in the rapid death of the cultures.

BIBLIOGRAPHY.

Hoven, H., Contribution à l'étude du fonctionnement des cellules glandulaires. Du rôle du chondriome dans la sécrétion, *Anat. Anz.*, 1910, xxxvii, 343.

Lewis, M. R., The formation of the fat droplets in the cells of tissue-cultures, *Science*, 1918, xlviii, 398.

Lewis, M. R., The formation of vacuoles due to *Bacillus typhosus* in the cells of tissue cultures of the intestine of the chick embryo, *J. Exp. Med.*, 1920, xxxi, 293.

Lewis, W. H., Degeneration granules and vacuoles in the fibroblasts of chick embryos cultivated *in vitro*, *Bull. Johns Hopkins Hosp.*, 1919, xxx, 81.

Lewis, W. H., Giant centrospheres in degenerating mesenchyme cells of tissue cultures, *J. Exp. Med.*, 1920, xxxi, 275.

MacCallum, W. G., Textbook of pathology, Philadelphia, 1916.

Mislawsky, N., Ueber das chondriom der pankreaszellen, *Arch. mikr. Anat., Ite Abt.*, 1913, lxxxi, 394.

Plato, P., Ueber die "vitale" Färbbarkeit der Phagocyten des Menschen und einiger Säugethiere mit Neutralroth, *Arch. mikr. Anat.*, 1900, lvi, 868.

Prigosen, R., The formation of vacuoles in connective tissue cells due to abnormal conditions, *Bull. Johns Hopkins Hosp.*, 1921, xxxii, No. 362 (in press).

Saguchi, S., Studies on the glandular cells of the frog's pancreas, *Am. J. Anat.*, 1919–20, xxvi, 347.

Scott, W. J. M., Experimental mitochondrial changes in the pancreas in phosphorus poisoning, *Am. J. Anat.*, 1916, xx, 237.

Shipley, P. G., The physiological significance of the reaction of tissue cells to vital benzidene dyes, *Am. J. Physiol.*, 1919, xlix, 284.

Swezy, O., Egg albumen as a culture medium for chick embryo tissue, *Biol. Bull.*, 1915, xxviii, 47.

EXPLANATION OF PLATE 51.

All the figures are camera lucida drawings of cells from the subcutaneous tissue of chick embryos; oil immersion lens and No. 6 ocular.

FIG. 1. A spindle cell containing al. granules from a 48 hour culture of an 8 day embryo in egg albumin.

FIG. 2. A flat cell containing al. granules from a 48 hour culture of a 7 day embryo in egg albumin.

FIG. 3. A flat cell containing al. granules; a few degeneration vacuoles may be seen in the region of the centrosphere. 48 hour culture of an 8 day embryo in egg albumin.

FIG. 4. Mitosis of a cell containing al. granules.

FIG. 5. A cell from a 24 hour normal culture which had egg albumin on it for 72 hours.

FIG. 6. A cell from a 4 day culture in diluted egg albumin. This cell does not contain al. granules.

FIG. 7. A degenerating cell from a 4 day culture in diluted egg albumin.

FIG. 8. A cell from a culture of an 8 day embryo in egg albumin which had been stained with trypan blue.

FIG. 9. A cell undergoing mitotic division from a culture of an 8 day embryo in albumin from an egg incubated for 12 days.

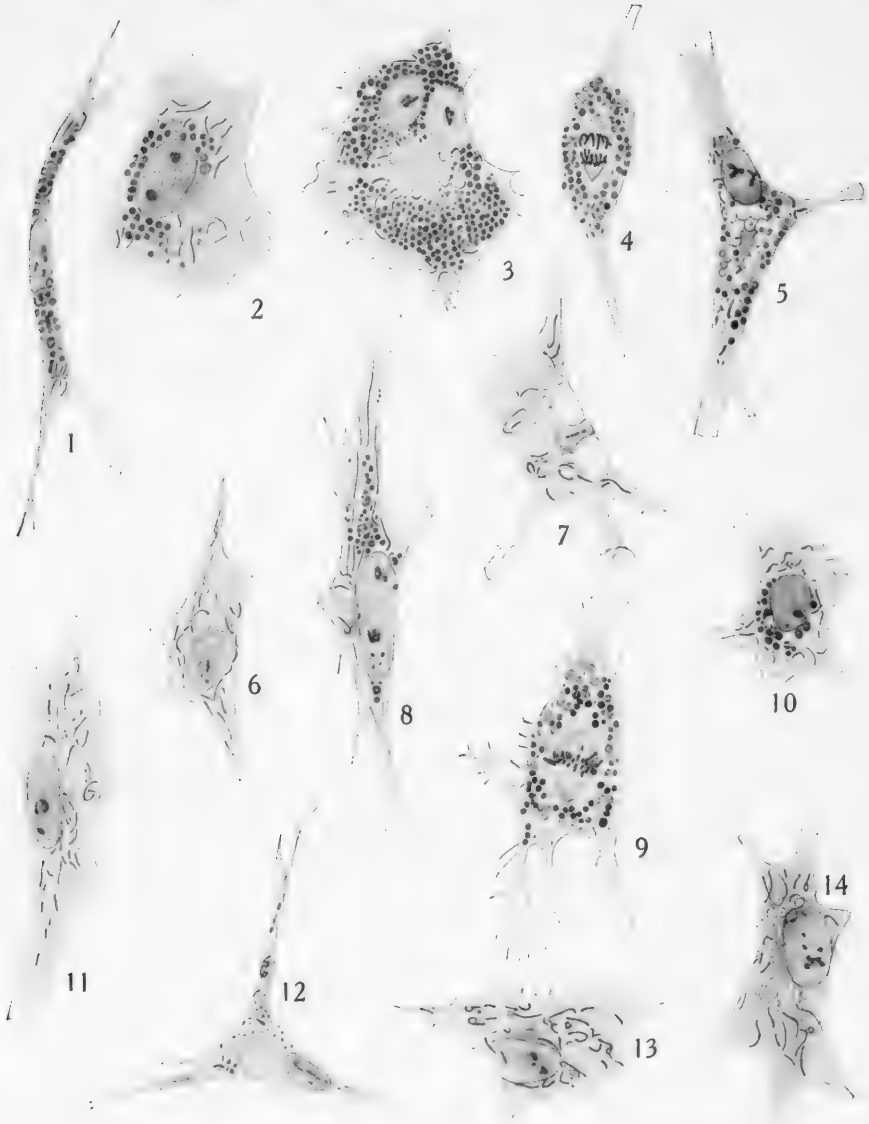
FIG. 10. A cell from a culture of *Fundulus* embryo in egg albumin.

FIG. 11. A cell from a 10 day culture of an 8 day embryo in Locke-Lewis solution.

FIG. 12. A cell containing a giant centrosphere from a culture which had the Locke-Lewis solution replaced by egg albumin for 24 hours.

FIG. 13. A cell free from al. granules which had 1 per cent Bacto peptone on it for 24 hours.

FIG. 14. A cell from a culture which had 5 per cent Bacto peptone placed upon it. This cell contains no al. granules but has degeneration vacuoles.



(Lewis: Tissue culture of chick embryos.)

EXPERIMENTAL SYPHILIS IN THE RABBIT.

VI. Affections of Bone, Cartilage, Tendons, and Synovial Membranes.

Part 1. Lesions of the Skeletal System.

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Plates 52 to 60.

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Syphilitic lesions of the skeletal system of the rabbit were first reported by Uhlenhuth and Mulzer (1) in 1910 under the rather indefinite term of "nose tumors." The conditions described by them were produced by intracardial and intravenous injections of massive doses of *Treponema pallidum* in young rabbits and appear to have been chiefly tumor-like swellings of the nasal mucosa and the soft tissues about the end of the nose. However, periostitis was described in one instance, and subsequent reports (2) contained illustrations of several types of the "nose tumors," which were obviously lesions of the nasal bones and cartilages.

Reasoner (3) also has mentioned the occurrence of a periosteal lesion of the nasal bones and refers to this type of affection as a late manifestation of the infection produced by certain strains of *Treponema pallidum*. None of these authors indicates the relative incidence of this class of lesions, and these are the only references to lesions of the skeletal system which have come to our notice. Apparently no instance of such a condition has been reported following a local inoculation, unless it be the case referred to by Reasoner.

In our experience, however, localized infections of the skeletal system, including the bones of the feet and legs and face, were quite common, hence it seems that the conditions have passed unrecognized and are, therefore, essentially new additions to the subject of experimental syphilis.

Among our earlier animals, there were 33, or 26 per cent, of those showing manifestations of generalized syphilis, in which lesions of the periosteum, bone, cartilage, tendons, and tendon sheaths were recognized by simple palpation and inspection. With the introduc-

tion of methods intended to increase the incidence and the severity of the generalized infection, as indicated in a previous paper (4), there was a marked increase in this particular group of conditions and the total number of cases available for study was more than doubled. In addition, the use of the radiograph in the diagnosis and study of deep seated lesions has been of considerable advantage.

The majority of the lesions seen were instances of periosteal, perichondrial, or bone involvement; only a few cases were recognized clinically in which the lesions appeared to originate in tendons, tendon sheaths, and like structures, and in a few instances affections of the joints were noted which appeared to be attributable to syphilitic infection. The parts chiefly affected were the facial and cranial bones and cartilages, and the bones, tendons, and joints of the feet, legs, and tail.

All these are lesions of hidden parts and in order to convey some idea of the nature of the processes concerned, it will be necessary to preface the clinical description of this form of experimental syphilis with a brief description of the lesions themselves.

General Character of the Lesions.

Lesions of the skeletal system proper occurred both in the periosteum and in the deeper parts of the bone or cartilage but most of the cases which came under our observation were clearly instances of primary periosteal involvement.

Periosteal Lesions.—These were of two types, the more common one being a nodular, granulomatous condition and the other a process of a more diffuse character. The first was characterized by the formation of small flattened or oval plaques, or of elevated nodules of extreme hardness distributed over the surface of the bones. Some of them spread laterally, forming masses which could barely be detected, while others became more elevated and reached a size of from a few millimeters to more than a centimeter in diameter.

Clinically, these lesions were readily distinguishable from cutaneous affections by the fact that the skin was freely movable over them and they were firmly attached to the underlying bone. When exposed to view, the earlier lesions appeared translucent and of a pale, slightly

yellow color, or presented an opalescent appearance and were surrounded by a faint areola of newly formed vessels. As the lesions developed, they became more dense and changed to a gray or yellowish gray color. Some were highly vascular and showed irregularly distributed areas of congestion and hemorrhage together with foci of necrosis. These conditions may be illustrated in general by Fig. 1, which is a photograph of an autopsy specimen showing the condition present 11 days after the lesions were first noted.

Upon section, some of the lesions were dense and fibrous, while others were more succulent or of a fleshy character. The cut surface of the larger ones frequently presented a mottled appearance due to the presence of irregularly distributed areas of necrosis and hemorrhage. In other cases, the surface was thickly stippled with minute gray or yellow points of necrosis or the entire center of the lesion was necrotic and demarcated from an outer zone of living tissue by the presence of a gray or yellowish gray line (Figs. 2 and 3).

The necrotic portion of the lesion remained firm and elastic, as a rule, but in exceptional instances or in cases of extensive necrosis, the central portion broke down into a softened or a cheesy mass (Fig. 3).

At the beginning, lesions of this type were largely confined to the periosteum, but as the process advanced, they frequently extended to the underlying bone. The changes in the bones themselves were quite variable. In general, they consisted of erosion of the outer table, or of bony caries, and finally led to an osseous overgrowth or the formation of osteophytic nodes. The destructive changes are shown in Figs. 4 and 5.

The first photograph (Fig. 4) shows the condition found at autopsy in an animal with actively progressing lesions of the facial bones. There were three groups of lesions present, the main mass occupying a position over the bridge of the nose while smaller lesions were present on both sides at a slightly higher level.

Upon turning back the flap of periosteum covering the left side of the nose (Fig. 5), the upper lesion appeared as a thickening of the periosteum corresponding with an area of erosion in the bone beneath. Over the bridge of the nose, there was a rather large granulomatous mass, most of which was firmly united with the periosteum. In the position occupied by this mass, a considerable area of the nasal bone had been completely destroyed, exposing the periosteum of the under side of the bone which was also affected. These lesions had been recognizable for only 19 days and yet the degree of destruction was quite marked.

Pathologically, the diffuse periostitis differed from the more circumscribed or nodular form chiefly in the presence of a thin layer of rather soft and friable tissue which covered a wide area of bone. During the earlier stages of the infection, little or no alteration could be detected in the contour of the part, but as the lesions increased, slight thickenings or irregularities could be made out.

On the whole, this type of process appeared to be more destructive than the circumscribed or nodular form; that is, its extension was lateral and inward rather than upward or outward. These lesions also tended to produce a chronic fibrous thickening of the periosteum such as that shown in Figs. 6 and 7. The thickening of the periosteum is here most apparent near the end of the nose where a bulbous enlargement has been produced. This was due in part to periosteal thickening and in part to a thickening of the bone and nasal cartilages (Fig. 7).

With bones such as the nasals, periosteal lesions developed from the under side of the bone as well as from the outer side. This is shown in a low power magnification of a cross-section of the nasal bone reproduced in Fig. 8.

Perichondrial lesions, especially those about the face, presented essentially the same characteristics as those of the periosteum. During periods of active growth, spirochetes could always be demonstrated in these as well as other bone lesions to be described, and were usually present in very large numbers.

Lesions in the Bone and Marrow Cavities.—In addition to primary periosteal involvement with subsequent extension to the bone, a number of lesions were seen which originated within the bone or marrow cavities. This class of infections, including what might be designated as osteitis, osteomyelitis, and epiphysitis or osteochondritis, is still somewhat obscure owing to the difficulty in detecting the lesions at an early stage of their development.

Clinically, little could be told of these affections by ordinary methods of examination until they had reached a fairly advanced stage. Necrosis of such bones as the nasals could then be detected by a crackling or giving of the bone beneath the palpating finger, and as the process extended to the outer surface of the bone, a deformity was produced which in most instances presented the same picture as that

of a primary periostitis. Lesions at the epiphyses of the long bones could also be detected in some instances by the presence of a swelling at the epiphyseal line, but they were difficult to distinguish from periosteal lesions which showed a marked tendency to localize at the same points.

By the use of x-rays, some early lesions were detected and their development was followed, but, as a rule, infection of the deeper parts of the bone was not discovered until the picture had been complicated by necrosis with dissolution of the bone or by pathological fracture. There are, therefore, two groups of conditions to be considered—one including lesions of obvious syphilitic origin and the other affections of a more obscure character.

By radiographs and by pathological examination, lesions were found in both the long and the flat bones. These appeared as focalized processes composed of a pale, translucent, and almost gelatinous material which spread out in the marrow cavities to a greater or less extent and invaded the substance of the bone. A lesion of this type is shown in Fig. 9 which is taken from the so called fifth metatarsal. As may be seen, there were in this case both periosteal and endosteal lesions as well as focal lesions within the bone itself. That the periosteal and endosteal lesions were not parts of the same process could be determined by differences in their histology, but such distinctions could not be made with lesions within the bone.

The internal lesions appeared to arise chiefly from the membrane lining the marrow cavity (endosteum) or in the loose perivascular tissues surrounding the larger vessels. In the long bones, they also showed a predilection for lines of epiphyseal union as shown in Fig. 10. This represents a fully developed lesion which originated in the epiphysis and subsequently spread to other parts of the bone.

Radiographs of the early lesions showed mainly a rarefication in the bone and a loss of the finer details of structure. These changes may be seen in Fig. 11, which shows two very distinct areas of involvement. One of these is in the shaft of the fifth metatarsal of the right foot (*a*) and the other at the proximal end of the corresponding bone of the left foot (*b*). The deformity of the right tarsus and their regularity seen in the calcaneus (*c*) were the result of an older lesion of a type to be described later.

This class of infections naturally gave rise to pathological alterations in the bone, which were characterized by necrosis with a more or less gradual disintegration of the bone or by increased fragility (when necrosis was more rapid) and the occurrence of pathological fracture or epiphyseal separation.

With these facts in mind, attention may be called to another group of bone lesions whose early stages are very difficult of recognition. These include chiefly cases of necrosis, fracture, and epiphyseal separation of the calcaneus with, occasionally, similar lesions in other bones, and may be illustrated by the affections of the tarsus shown in Figs. 12 to 14.

The history of these affections was much the same in all cases; namely, the sudden development of lameness, with a marked edematous swelling of the entire tarsus together with some tenderness and the presence of a crepitus. The first cases which were noticed were regarded as traumatic injuries and no particular attention was paid to them until it was found that similar conditions occurred as a result of obvious syphilitic infection.

A more careful investigation was then attempted and the nature of the injury to the bone determined by both radiographic and pathological examination. Spirochetes could not be found in fluid aspirated from these cases[1] and the condition was complicated by the reaction incident to the sudden disruption of the bone.

From an examination of serial radiographs of several of these animals, abnormalities of the bones could be made out in some which antedated the occurrence of the lesion in question. In Fig. 15, a peculiar defect is seen in the calcaneus of the right foot. 19 days later, this bone gave way in the middle and anterior portions, permitting the talus to sink downward as shown in Fig. 16, the details of the bony structures being masked at this time by an effusion into the tissues. There was also a dropping of the arch.

A second case is shown in Figs. 17 and 18. The first point to be noted is the dissimilarity of the posterior ends of the calcanei. The left bone shows a narrowing of the neck which extends well below the level of the tuberosity, while on the right, there is even some fullness in this region. This bone shows a band of lighter

[1] The demonstration of spirochetes in such cases by animal inoculation would be of no value, since the animals were known to have a generalized infection capable of transmission by blood inoculation.

shadow in the position of the epiphyseal line. 8 days later (Fig. 18) there was an epiphyseal separation, or fracture, which followed this line quite closely. Other examples of a similar character were also seen.

A significant feature of this class of lesions is that among the large number of animals, infected and uninfected, which were handled during the course of this work, with one possible exception, lesions of this type occurred only in animals with obvious syphilitic lesions of other bones (note metatarsals in Figs. 11 and 12). It may be mentioned also that definite periosteal lesions of the anterior end of the calcaneus have been noted, and while no gross destruction of bone was detected in these instances, all the obscure cases of necrosis such as that in Fig. 12 have occurred in exactly the same position as that occupied by the periosteal lesions.

Histologically, however, the extent of bone involvement always proved to be greater than the gross appearance would indicate, and bones which appeared to be very little affected in the gross frequently proved upon histological examination to be honeycombed by the syphilitic process. Further description of these lesions will be found below in the section on the histological changes observed in various types of bone lesions.

Lesions of Tendons and Tendon Sheaths.—There is no record of the occurrence of syphilitic lesions of the tendons or tendon sheaths of the rabbit so far as we are aware, and the number observed by us was comparatively small.

The typical lesion in cases of primary tendon involvement was a circumscribed granulomatous process of essentially the same character as that of the periosteum, and with a few exceptions the lesions were located in the tendo achillis or its sheath. The exceptions noted were small multiple lesions involving the tendons on the dorsum of the carpus and front feet and the dorsum of the hind feet. Some of these were not recognized until the animal came to autopsy, and it is possible that other cases of a similar character might have escaped our notice.

Secondary involvement of tendons and ligaments as a result of direct extension of lesions originating elsewhere was of comparatively frequent occurrence. This was not unusual with periosteal lesions

about the carpus and tarsus and the small bones of the feet, and with cutaneous lesions of the tail and lateral surfaces of the hind feet. Fig. 2 furnishes a good illustration of this type of condition.

Diffuse Exudative Reactions Associated with Lesions of the Bones.

In addition to the lesions described, an acute exudative reaction affecting the parts immediately surrounding a focus of bone affection may be referred to briefly. This reaction rarely occurred except with lesions of the tarsus or of the small bones of the feet. In cases of infection of the metatarsals and the phalanges, there was frequently an exudation into the surrounding tissues which was composed chiefly of serum with a few leucocytes and polyblasts. Such reactions occurred most often where there was a considerable degree of bone destruction.

Similar conditions have already been referred to in connection with lesions of the tarsal bones. In addition, there were a few instances in which the reaction occurred where no bone lesion was demonstrable. The inciting factor in these cases is not known, but the subsequent history of the condition, with the development of a diffuse fibrosis and fixation of the affected parts, suggests the possibility of an involvement of synovial membranes of tendon sheaths and joint cavities. Histologically, it has been shown that the *pallidum* infection may become localized in such structures, but whether this takes place by direct extension from other lesions or as an independent focus of infection is as yet undetermined.

Histology.

Histologically, the details of the pathological process concerned in syphilitic infections of the bones are too numerous and too complex to be described in the present connection. As a means of orientation, however, it seems well to refer briefly to certain features of the lesions found during active stages of the infection. Three fairly well defined groups of conditions can be recognized: (1) a granulomatous process which is not unlike that of other syphilitic lesions; (2) a condition which is characterized chiefly by absorption or by necrosis and disintegration of bone; and (3) a lesion which combines the processes

of granulomatous proliferation, absorption, and necrosis with osseous overgrowth. This last condition will not be considered at the present time.

The granulomatous lesions usually seen in cases of bone syphilis may arise from any part of the bone and present certain structural peculiarities. In general, they are composed of a matrix of newly formed connective tissue with a network of capillary vessels; there are the usual polyblastic infiltration and a marked tendency on the part of these cells to assume a perivascular arrangement. Polynuclear giant cells are also present and are frequently very numerous, especially where marked bone destruction occurs. The picture presented in these cases is of the type of an active foreign body reaction or may even suggest that of the so called giant cell osteosarcoma.

When the lesion arises from the periosteum, one frequently finds an interesting structural arrangement of the elements which participate in the reaction (Figs. 19 and 20). There are two or three distinct layers which correspond roughly with structural divisions of the periosteum. There is first an outer cellular layer which is composed of loosely arranged connective tissue cells and polyblasts occupying the position of the loose areolar tissue covering the outer surface of the periosteum (Figs. 19 and 20). Then comes a denser layer of fibroblasts which contains numerous focal and perivascular accumulations of round cells and corresponds to the dense fibrous layer of the periosteum. Finally, either as a part of this second stratum or as a distinct layer in itself, there is a zone which corresponds with the osteogenetic layer of the periosteum. At first, this is composed of a row of giant cells (osteoclasts) whose processes dip downward into the bone, and a small amount of newly formed connective tissue infiltrated with polyblastic cells. This is the osteoclastic layer of the lesion and its distinctive elements, the osteoclasts, are derived from the osteoblasts of the periosteum.

The composition of periosteal lesions differs very decidedly in different classes of bones. Thus, in bones which are preformed in membrane, such as the nasal bones, a two layered structure prevails (Fig. 19). This is composed of the loose outer layer and a highly developed third or osteoclastic layer, while the second or fibrous layer is usually absent or but slightly developed. The lesions of the long bones

(cartilage bones), on the other hand, show three layers, the most highly developed of which is the middle or fibrous layer. In addition, the osteoclastic layer is more fibrous and shows relatively few giant cells. These facts are of importance in interpreting the pathological alterations produced in different classes of bones.

The growth of some periosteal lesions is marked by an increase in the thickness of the outer layers, of others by a peripheral extension of all three layers, or by a downward growth of the inner layer. In the first instance, the condition produced is a prominent periosteal nodule, or granuloma, in the second a diffuse periostitis, and in the third marked destruction of the underlying bone. It is in instances of the last group that the osteoclastic layer of the lesion becomes so highly developed; in membrane bones, the greater part of the lesion in such cases presents the picture of the giant cell granuloma, but this is not true of bones which are preformed in cartilage.

Lesions arising within the bone show essentially the same composition as those from the periosteum, except for their structural arrangement, and are, therefore, difficult to distinguish from them. Endosteal affections, on the other hand, are almost devoid of fixed connective tissue elements. They are composed of a loosely arranged stroma which is thickly infiltrated with polyblasts and contains a network of capillary vessels. The lesion is, therefore, more of an infiltrative than of a granulomatous process.

The alterations observed in the bone itself were quite variable. In some instances, the bone presented a practically normal appearance except for the presence of irregularities or a honeycombing of the bone due to gradual absorption, and in some, even small fragments of bone showed a well preserved architecture (Fig. 21). Frequently, however, there were mass necrosis and rapid disintegration of bone extending over wide areas. The picture presented in these cases (Fig. 22) was very striking and consisted of necrosis of bone corpuscles and reduction of the ground substance to a thin and ragged framework of granular or fibrillated material which stained very faintly if at all. The impression created was that of large masses of bone undergoing solution, and various stages in the process could be traced down to the last shadowy framework of fibers and granules (Fig. 22).

Emphasis may be placed upon these processes of bone destruction on account of the occurrence of similar changes in portions of the bone where little or no syphilitic reaction of the generally accepted type could be made out. This was especially noticeable in the lesions of the calcaneus described above. There were two forms of alteration to be noted. In one group of cases, the alteration consisted of a simple bone absorption with reduction in the density and the volume of the bone; in a second, there were necrosis and disintegration.

The first group developed by widening out of the lacunæ and of the perivascular spaces, which became filled with a loose and rather cellular connective tissue. The compact portion of long bones such as metatarsals and phalanges thus tended to become somewhat cancellous in structure, while the cancellous portion of the bone was distinctly reduced in amount. As a result of these changes, there were a reduction in both the density and the thickness of the bones and a marked enlargement of the marrow cavity. Normally, these bones contain some cellular marrow, but in affections of the type described, there were not only bone absorption but a conversion of a cellular (hemopoietic) to a fatty marrow which, in some cases, was complete.

Mass necrosis of portions of the bone which were apparently not directly involved by the granulomatous lesions needs no further description than that given above.

The most obvious explanation of these conditions is one based upon disturbed nutrition. There is little doubt that the primary seat of injury in all syphilitic processes is the endothelial cell, and while those in the immediate vicinity of active syphilitic lesions suffer more than do others, it appears that the effects of the toxic products which are elaborated may reach every such element of the body. Thus, while the smaller vessels in the immediate vicinity of a lesion are most affected, it would seem practically certain that in pronounced cases of syphilitic infection, the entire capillary mechanism is subjected to injury the degree of which varies with the individual case.

If this is applied to the bone lesions described, both the absorption type of phenomenon and the mass necrosis might be accounted for upon the basis of a reduction in the blood supply due to capillary injury in the first case and to a more extensive vascular injury in the second. It would be going too far to assume, however, that other

factors had no part in the production of these lesions, but the reduction of blood supply due to vascular injury appears to be the simplest and most immediate explanation which can be offered.

The fact that rarefication and necrosis of bone are not necessarily parallel with the size or extent of the granulomatous lesion but may occur even in the absence of any marked granulomatous reaction is of considerable importance, and the obscure cases of necrosis and fracture of the calcaneus described above were largely due to this type of bone lesion.

Histologically, small granulomatous lesions which resemble those of the periosteum or endosteum in all essential respects have been found in the synovial membranes of joint cavities, notably those of the tarsus and the metatarsophalangeal joints. In these cases, there was infection of neighboring parts, and involvement of the joints appeared to have taken place by extension from adjacent foci of infection. While it appears probable, therefore, that primary localization in the joint may occur, this has not as yet been demonstrated.

Gross Alterations in the Bones.

Frequent reference has been made above to various types of bone injury resulting from infection of the periosteum or of the bone itself. Before concluding the description of this group of lesions, it seems well, however, to give a more definite statement as to the gross alterations which are produced.

Nasal Bones and Splints.—The most marked and characteristic lesions were those of the nasal bones and splints. The effects produced may be illustrated by the series of photographs reproduced in Figs. 23 to 30. These photographs were taken from dried skulls and are intended to show alterations produced by focal and diffuse lesions during active stages of the infection as well as the results of repair.

Focal lesions of the nasal bones, as shown in Figs. 23 to 26, may produce anything from the slightest surface erosion or roughening to a complete and sharply circumscribed defect in the bone. All of the group here shown were taken toward the end of the infection and may be regarded, therefore, as representing the maximum of bone destruction produced in each instance. In Fig. 23, there was only a surface erosion, while in the second animal of the series (Figs. 24 and 25) the

necrosis extended much deeper, forming a partial defect in the bone. This defect was easily recognizable during life, and at autopsy it was found that there was little left of the bone except a thin layer of spongy necrotic fragments loosely attached to the underlying membranes. The effect produced in this case might be compared to the saddle-nose deformity of man.

The lesion involving the upper right nasal and the maxillary process of the frontal bone in Fig. 26 shows complete necrosis and removal of the bone, resulting in the formation of a small but very sharply circumscribed defect. It should be stated that the clean-cut appearance presented by this lesion was probably due to the fact that it was of internal rather than external origin. There was a very slight external growth in this case, and when this first appeared, necrosis of the bone had already taken place.

The conditions represented in Figs. 27 and 28 differ from those preceding only in that they represent processes of a more diffuse character, which, as has been explained, are usually more destructive in their effects.

Figs. 29 and 30 represent processes of repair. The lesion in Fig. 29 was of the same type as that in Figs. 27 and 28, but led to complete necrosis of a large part of both nasal bones. The condition here shown is the regeneration effected during a period of about 7 weeks. The bones were considerably thickened throughout; in the upper portion, regeneration was very imperfect, and while the bone towards the end of the nose was of a decidedly spongy character, it was much more nearly normal than that above and the probability is that in time the entire area would have assumed this appearance.

This case illustrates a remarkable tendency on the part of the rabbit to an obliteration of the marks of syphilitic lesions in the bones. Small erosions and defects of the nasal bones are usually repaired so as to leave little if any evidence of the previous injury, and the permanent alterations produced by the most destructive lesions are much less than one might expect. This evidence usually consists of a thickened and adherent periosteum, slight irregularities in the bone, surface roughening and thickening of the bone (*cf.* Figs. 6 and 7), and the presence of slight depressions or bony prominences. The last conditions are occasionally fairly well marked, as indicated by the node on the nasal splint in Fig. 30.

Anomalous Conditions of the Parietals and Occipitals.—Before leaving this phase of bone syphilis, attention may be called to the condition of the parietal and occipital bones shown in Fig. 31. The cause of this condition is not known, but, as may be seen, there is a decided thickening of the bones which are very porous or spongy in character.

Various degrees of this condition have been observed at autopsy in a number of animals. Most of them were instances of long standing syphilitic infection; some animals were in excellent physical condition, while others showed obvious signs of malnutrition for which no cause could be found. In some, the bones contained an abundant red marrow; in others, the marrow was more fatty in character.

It is, of course, well known that similar changes in these bones may be produced through the development of an anemia, and there is at present no reason to regard the processes here shown as the result of a local infection. The circumstances suggest, however, that they may be produced in response to a systemic condition resulting from the syphilitic infection.

Ulna, Radius, and Tibiofibular.—Many of the long bones such as the ulna, the radius, and the tibiofibular show little alteration in appearance at any stage of the infection. Usually there was no more than a slight roughening of the surface of the bone with either an increase or a decrease in thickness. These areas also showed a honeycombing of the bone, but the apparent alteration was always less than that shown by microscopic examination.

The tendency was also towards a rapid restoration to normal, and bones examined a few months after the lesion had resolved showed little if any abnormality.

Metatarsals and Phalanges.—Gross changes in the metatarsals and the phalanges were more marked. During the active stages of the infection, there were usually surface erosion, necrosis, and disintegration, epiphyseal separation, or fracture. Less often, osseous overgrowth or the laying down of considerable masses of soft, spongy bone occurred, while the lesion was still actively progressing (Fig. 10).

As the infection subsided and the injury to the bone was repaired, various abnormalities were observed. These consisted of changes in the form of the bone, surface roughening, and honeycombing, together with irregular reductions or increases in thickness such as were illustrated for the nasal bones. Many of these changes appeared to be of a semipermanent character. The abnormality diminished with time, but in a few animals which were held for observation, marks of the previous injury were still recognizable several months after the infection had subsided.

Tarsus.—Deformities of the tarsus following necrosis, fracture, or epiphyseal separation of the calcaneus were always marked. These conditions invariably led to excessive bone formation. Necrosis and disintegration of the calcaneus were followed by the formation of an irregular granular mass of bone such as that shown in Fig. 32. The union of a fracture or of an epiphyseal separation, on the other hand, was accomplished by the laying down of an excessive amount of callus and the formation of a large mass of spongy bone (Fig. 33). These conditions were also of a more or less permanent character. The absorption of callus and the reestablishment of compact bone took place slowly—more slowly than after ordinary trauma.

SUMMARY.

From a study of a series of rabbits inoculated with two old strains of *Treponema pallidum*, it was found that localized infection of bones and tendons was of frequent occurrence and led to the formation of a variety of lesions.

The bones usually involved were those of the face and the feet and legs. Most often the lesions arose from the periosteum but developed also within the bone or marrow cavities and at lines of epiphyseal union.

Grossly, the periosteal lesions were of two types—one being a circumscribed, indurated, and nodular mass and the other a process of a more diffuse character. Histologically, the lesions presented the typical appearance of syphilitic granulomata composed of more or less distinct layers which corresponded roughly with structural divisions of the periosteum. The composition of lesions of membrane and of cartilage bones differed somewhat in this respect, especially in the development of an osteoclastic layer. Invasion of the bone with absorption and necrosis were constant features of periosteal affections and were most marked in the case of the facial bones and the small bones of the feet.

Lesions in the bone and marrow cavities were detected chiefly by radiographs or by the occurrence of bone destruction in the absence of periosteal involvement. They were characterized by a loss of structural detail in the bone, rarefication, increased fragility, necrosis,

pathological fracture, and epiphyseal separation associated with more or less granulomatous reaction. Histologically, the bone lesions presented essentially the same picture as those of the periosteum, while the lesions which arose from the marrow cavities were composed chiefly of polyblastic infiltrations. In this group of affections, the most important were those which developed at the epiphyses.

The destructive effects produced by all classes of lesions varied from a slight surface erosion or rarefication to extensive necrosis resulting in the formation of bony defects or in disintegration or fracture of the bone. These conditions differed very decidedly with the particular bones involved.

Of especial importance in this connection was the occurrence of a peculiar form of mass necrosis which at times resulted in the destruction of considerable areas of bone even in parts where the granulomatous type of lesion was comparatively slight. The most characteristic injuries were the saddle-nose deformities and the epiphyseal separation in the small bones of the tarsus and hind feet.

The marks of permanent injury were, on the whole, comparatively slight, but they also differed both with the degree of the original injury and with the bone affected.

Granulomatous lesions of tendons or tendon sheaths were occasionally seen, and in a few instances, lesions of synovial cavities were demonstrated microscopically.

BIBLIOGRAPHY.

1. Uhlenhuth and Mulzer, P., *Arb. k. Gsndhtsamte*, 1910, xxxiv, 222.
2. Uhlenhuth, P., and Mulzer, P., *Arb. k. Gsndhtsamte*, 1913, xliv, 307; Atlas der experimentellen Kaninchensyphilis, Berlin, 1914.
3. Reasoner, M. A., *J. Am. Med. Assn.*, 1916, lxvii, 1799.
4. Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1920, xxxii, 445.

EXPLANATION OF PLATES.

The photographs and radiographs represent the objects at their natural size. None of the illustrations has been retouched. Statements of time, unless otherwise indicated, are estimated from the date of inoculation.

PLATE 52.

FIGS. 1 to 7. Nodular and diffuse periostitis.

FIG. 1. 74 days. Typical periosteal granulomata of the nasal bones, growth tending to be upward rather than inward.

FIG. 2. 123 days. Periosteal granuloma on the shaft of the fifth metatarsal and involving the tendons of the dorsum of the foot. The cut surface shows irregular miliary areas of necrosis.

FIG. 3. 137 days. Periosteal granulomata of the tibiofibular, the fifth metatarsal, and the phalanges. The lesion of the tibiofibular shows a central area of necrosis with softening and a mottling due to congestion and hemorrhage.

FIG. 4. 84 days. Multiple periosteal lesions of the nasal bones.

FIG. 5. The same specimen with the periosteum dissected back. The lesion on the upper part of the nasal bone is of slight extent and shows only a surface erosion of the bone; that over the bridge of the nose is composed chiefly of an osteoclastic growth, and there are complete necrosis and absorption of the bone in this area.

FIG. 6. 2 years and 5 months. Chronic fibrous periostitis of the nasal bones of a diffuse type. The main lesions appear as bulbous masses at the end of the nose.

FIG. 7. The same preparation with the periosteum removed from the left side. There is an irregular thickening of the bone which is most evident in the lower third, and the periosteum was firmly adherent at many points. Note also the irregularity of the end of the nasal bone.

PLATE 53.

FIGS. 8 and 9. Photomicrographs of periosteal and endosteal granulomata.

FIG. 8. 48 days. Cross-section of nasal bone showing periosteal and perichondrial lesion arising from the inner side of the bone and working its way outward. The external coverings of the bone are normal. × 95.

FIG. 9. 89 days. Proximal end of the fifth metatarsal. The photograph shows a combination of lesions, the chief one being a granulomatous nodule in the marrow cavity; there is a distinct widening of this part of the cavity. Lesions are also seen in the bone and on both periosteal surfaces. × 30.

PLATE 54.

FIG. 10. 89 days. An epiphyseal lesion of the distal end of the fifth metatarsal. There has been a separation of the epiphysis which apparently took place in the directions indicated by the arrows. At the time represented in the photograph,

this lesion was in process of repair but there was an active syphilitic granuloma in the epiphysis. A periostitis is also seen to be present and there is an extension of the infection to the synovial membranes lining the joint cavity. × 30.

PLATE 55.

FIGS. 11 to 14. Radiographs of lesions developing within the bones.

FIG. 11. 119 days. The rarefication seen at *a* and *b* was due to osteitis of the shaft and proximal end of the metatarsals. The deformity of the tarsus at *c* was due to necrosis of the calcaneus.

FIG. 12. 163 days. Necrosis of the anterior end of the right calcaneus (marked by arrow). There were lesions also at the proximal ends of both lateral metatarsals (marked by arrows).

FIG. 13. 77 days. Pathological fracture of the middle of the calcaneus with effusion into the tissues.

FIG. 14. 73 days. Epiphyseal separation of the right calcaneus. The left calcaneus also shows some degree of abnormality which is indicated chiefly by the thickening of the upper surface of the bone toward its posterior extremity. Normally the shadow of this portion of the bone forms a straight line (*cf.* other radiographs).

PLATE 56.

FIGS. 15 to 18. Radiographs showing lesions of the bone.

FIGS. 15 and 16. Successive stages in the development of a lesion of the right calcaneus. Fig. 15 (85 days) shows a peculiar defect in the lower portion of the calcaneus which is marked by an arrow. The left calcaneus is normal. Fig. 16, which was taken 19 days later, shows an outspoken deformity of the right calcaneus. This was due in part to a dropping of the arch from involvement of the ligaments and in part to necrosis of the calcaneus, the talus becoming embedded in its substance. Note the relative position of the talus in Figs. 15 and 16.

FIGS. 17 and 18. Stages in the development of an epiphyseal separation. In Fig. 17 (77 days) there is a broad band of rarefication at the epiphyseal line of the right calcaneus (marked by arrow) and a suggestion of a separation is seen at the top of this line. The posterior end of the left calcaneus also shows a narrowing of the neck which is abnormal. Fig. 18, taken 8 days later, shows a separation of the epiphysis of the right calcaneus which followed the line indicated in Fig. 17.

PLATE 57.

FIGS. 19 and 20. Photomicrographs showing characteristic periosteal lesions of membrane and cartilage bone.

FIG. 19. 60 days. Periostitis of the nasal bone showing an outer cellular layer and an inner osteoclastic layer. The cellular layer is composed chiefly of fibroblasts with a moderate degree of polyblastic infiltration. It is also quite vascular. The osteoclastic layer is composed of fibroblasts and polyblasts together with a large number of osteoclastic cells. × 95.

FIG. 20. 77 days. Periostitis of the metatarsal. The lesion shows three distinct layers which present much the same general appearance. The two outer layers show a considerable degree of polyblastic infiltration; they are more vascular than the inner layer and the vessels show well marked endarteritis. The osteoclastic layer is but slightly developed; it is largely fibrous in character and contains very few osteoclasts. × 95.

PLATE 58.

FIGS. 21 and 22. Photomicrographs, taken from the nasal bone, showing different types of bone destruction.

FIG. 21. 60 days. Necrosis and absorption of bone accomplished chiefly by surface action. Note the wave lines in the bone, the preservation of architecture, and the survival of bone corpuscles. × 135.

FIG. 22. 100 days. Mass necrosis with rapid disintegration of bone—a type of lesion frequently observed in cases of marked bone destruction as it occurs in an acutely progressive process. × 135.

PLATE 59.

FIGS. 23 to 26. Photographs of dried preparations showing the maximum bone injury produced by various types of lesions.

FIG 23. 121 days. Surface erosion of the nasal bones produced by a large periosteal granuloma, the growth of which was mostly in the outer layers.

FIG. 24. 118 days. Necrosis of the nasal bones with the production of saddle-nose deformity due to an invasive periosteal lesion.

FIG. 25. The same specimen viewed from the side.

FIG. 26. 124 days. A defect of the right nasal bone produced by a lesion arising from the inner side of the bone. There was only a suggestion of periosteal thickening in this area.

PLATE 60.

FIGS. 27 to 33. Bone lesions and the deformities produced in the process of repair.

FIG. 27. 123 days. A widespread necrosis of the nasal bones resulting from a diffuse periostitis which produced only a slight palpable thickening over the surface of the bone.

FIG. 28. 137 days. Similar lesion viewed from the side. Note especially the destruction of the margin of the bone, the involvement of the lateral splints, and extension of the lesion to the premaxilla.

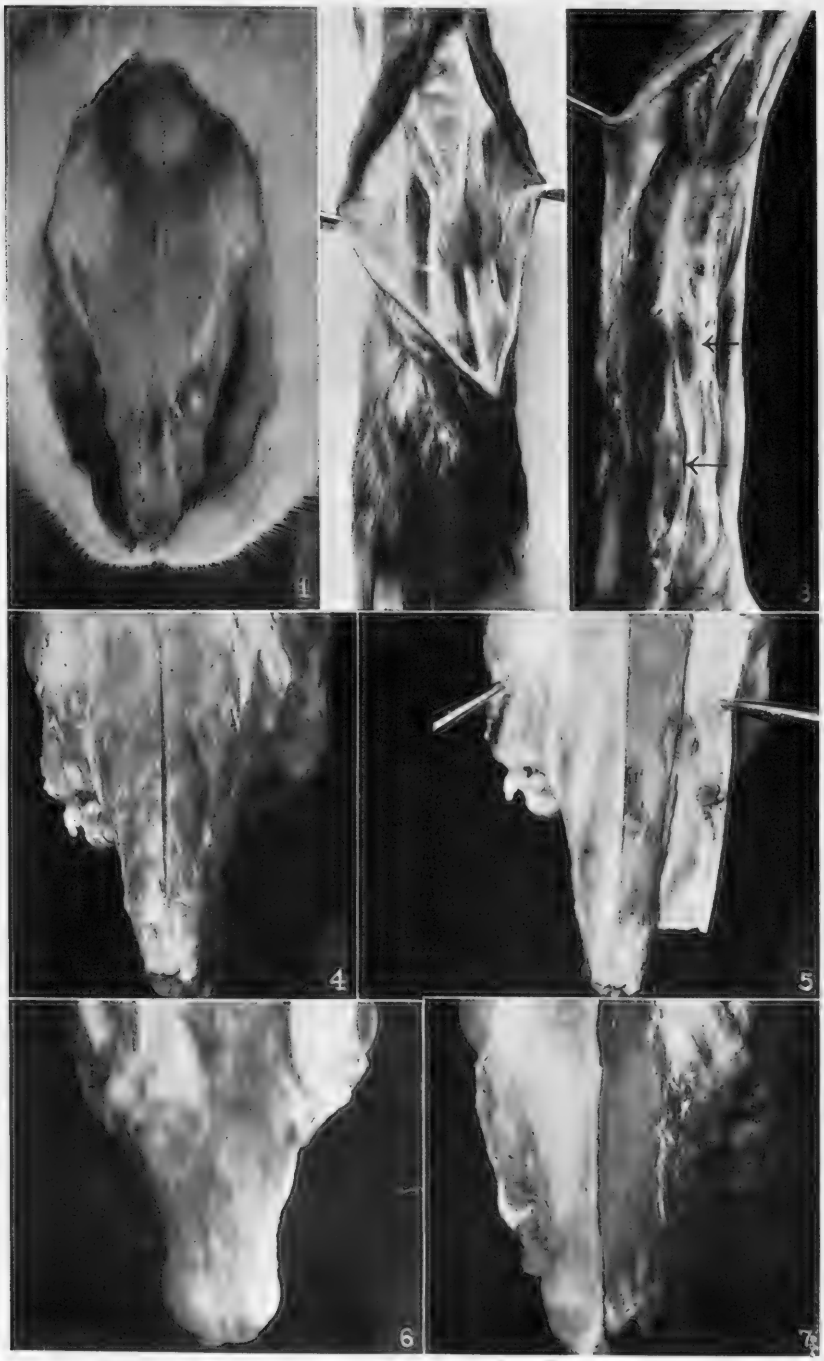
FIG. 29. 121 days. An imperfect regeneration of the nasal bones following almost complete necrosis of the areas involved. In the upper portion of the right nasal, the bone is very irregular, while below it is comparatively smooth but considerably thickened, and a defect is still present in this part of the bone.

FIG. 30. 130 days. An osteophytic node at the point of union of the frontal process of the premaxilla and the maxillary process of the frontal bone (nasal splints), illustrating a tendency of syphilitic lesions to become localized at points of bony union.

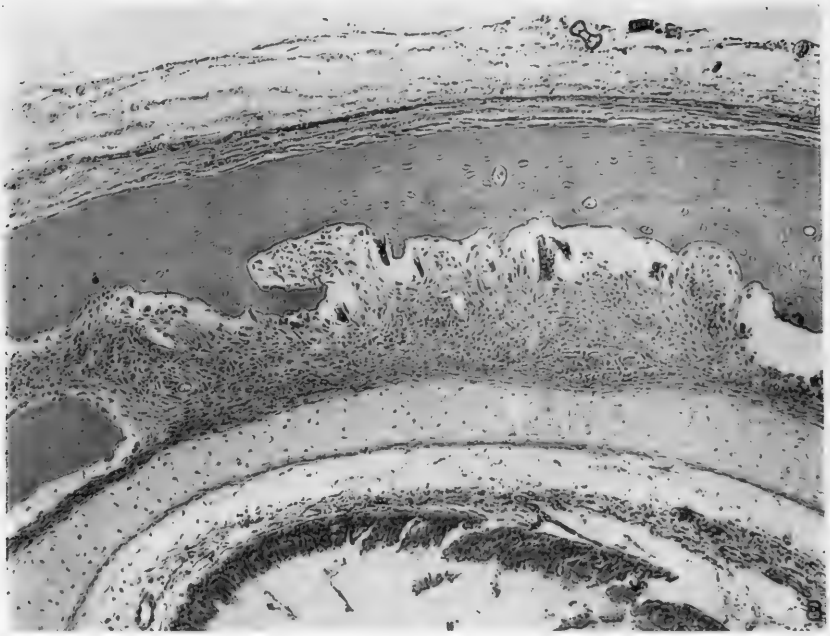
FIG. 31. 342 days. A thickened spongy condition of the parietal and occipital bones occurring late in the course of a generalized infection. The exact cause of the condition is not known.

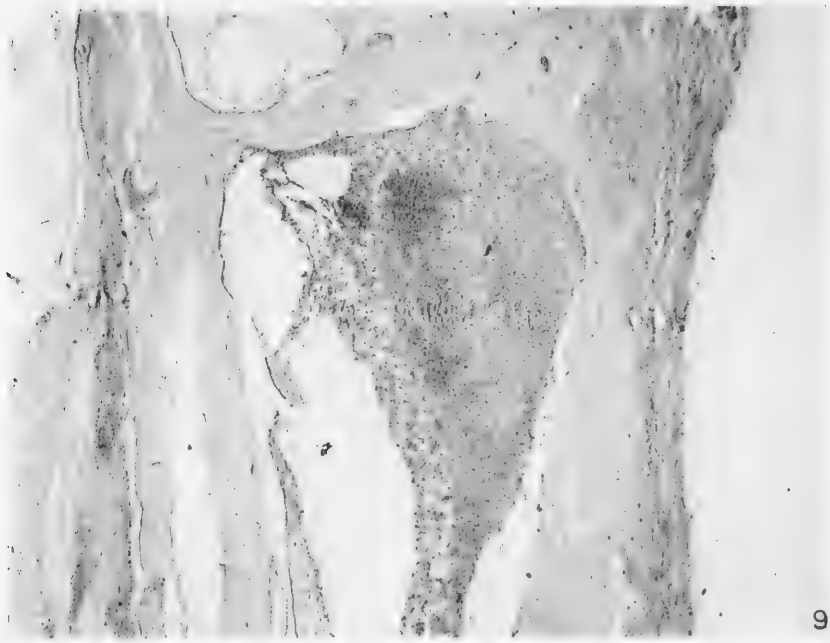
FIG. 32. 126 days. A deformity of the tarsus resulting from necrosis of the calcaneus. This bone is considerably thickened and extremely irregular.

FIG. 33. 126 days. A deformity produced by an epiphyseal separation of the calcaneus. The original lesion healed with the formation of a large mass of callus about the posterior end of the calcaneus and the separated epiphysis.



(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)





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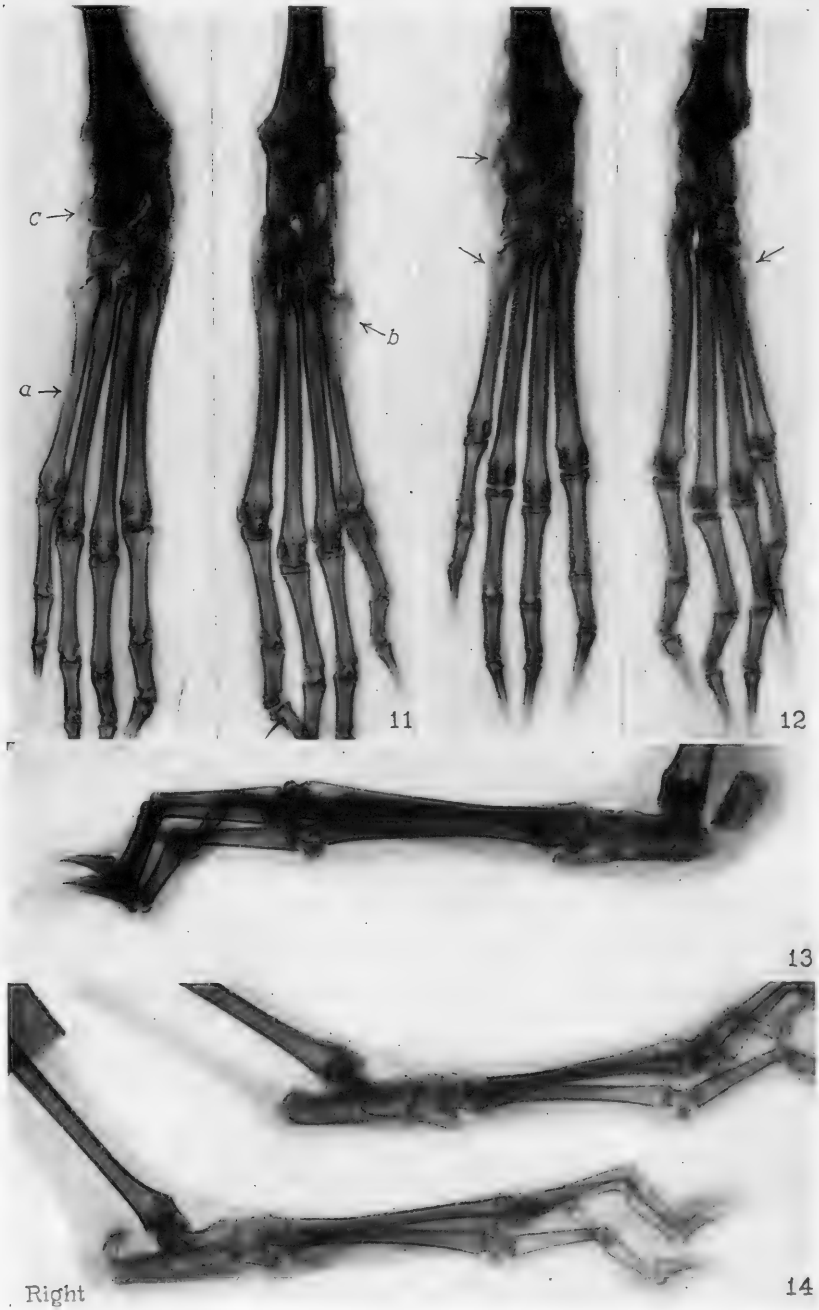

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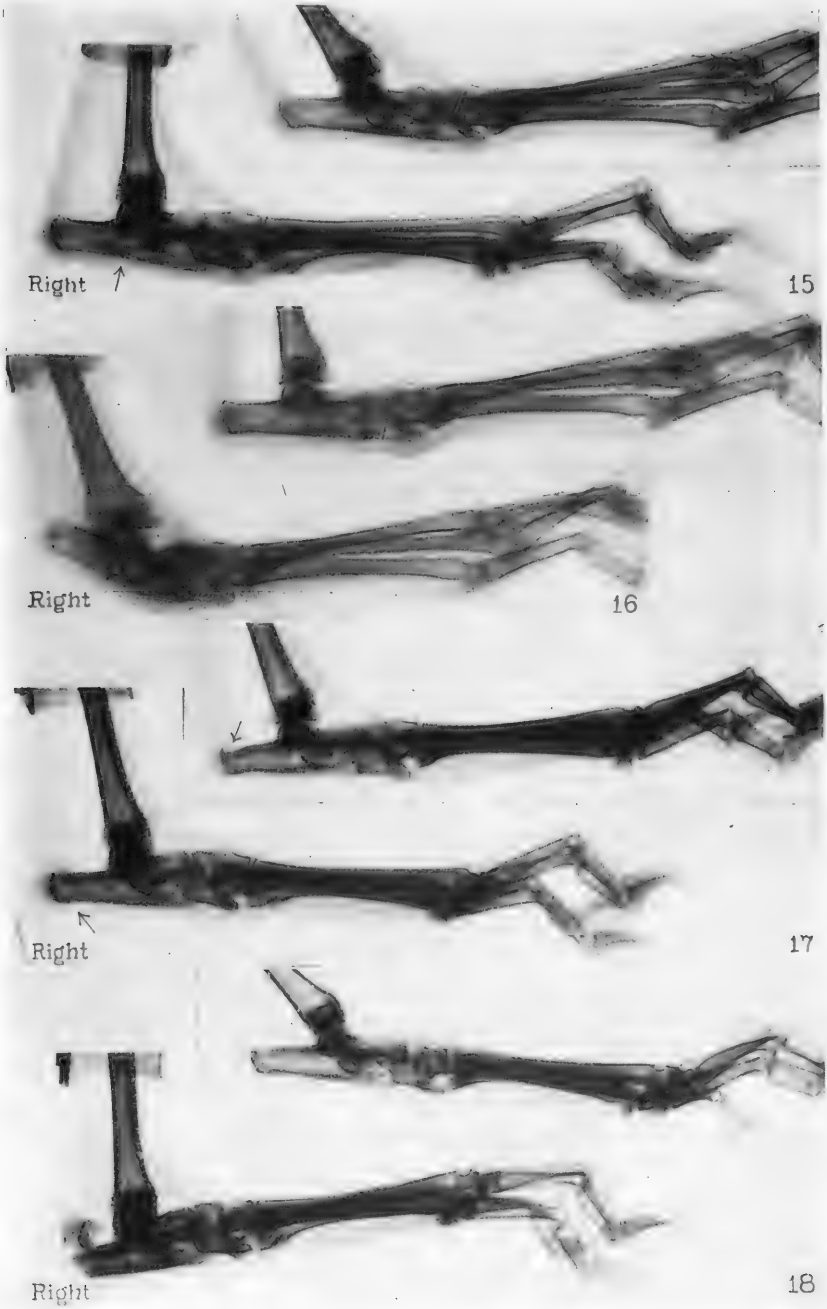
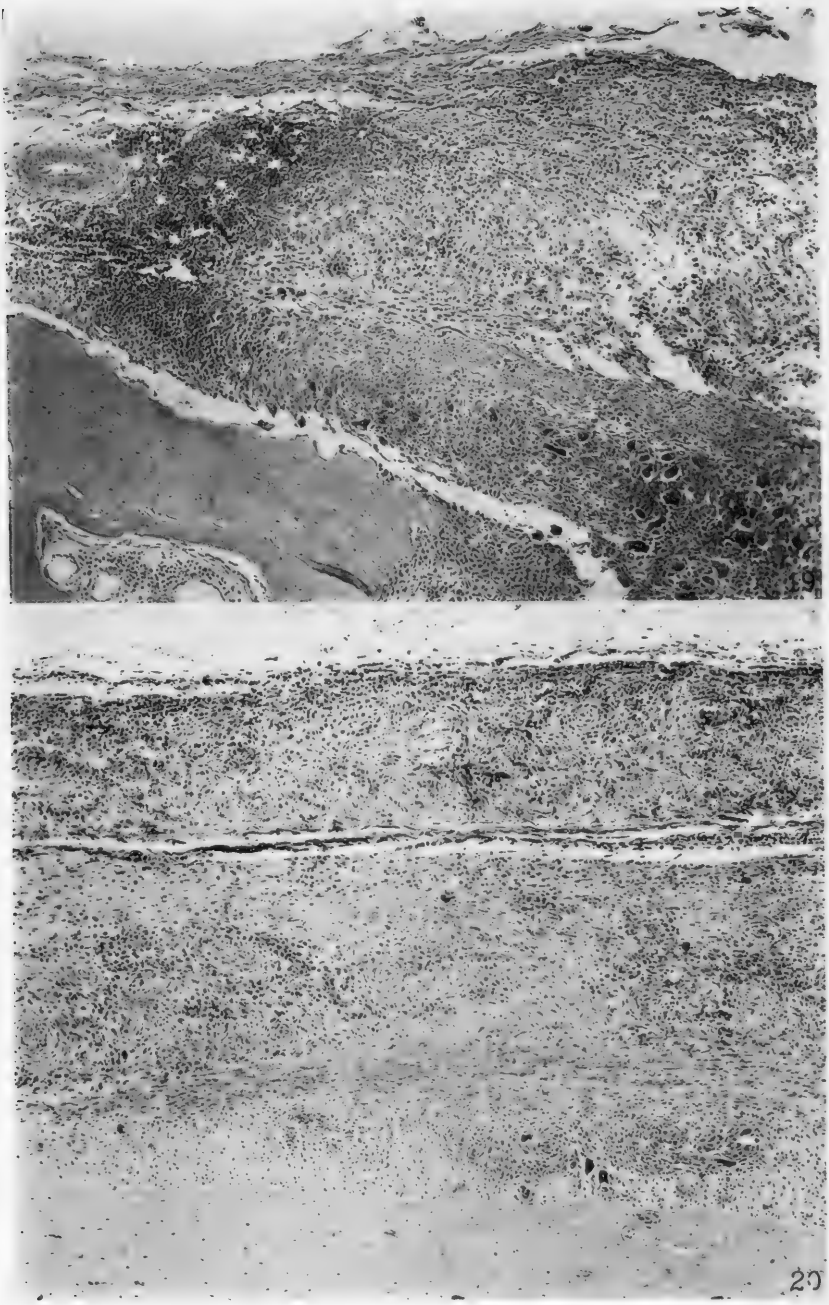
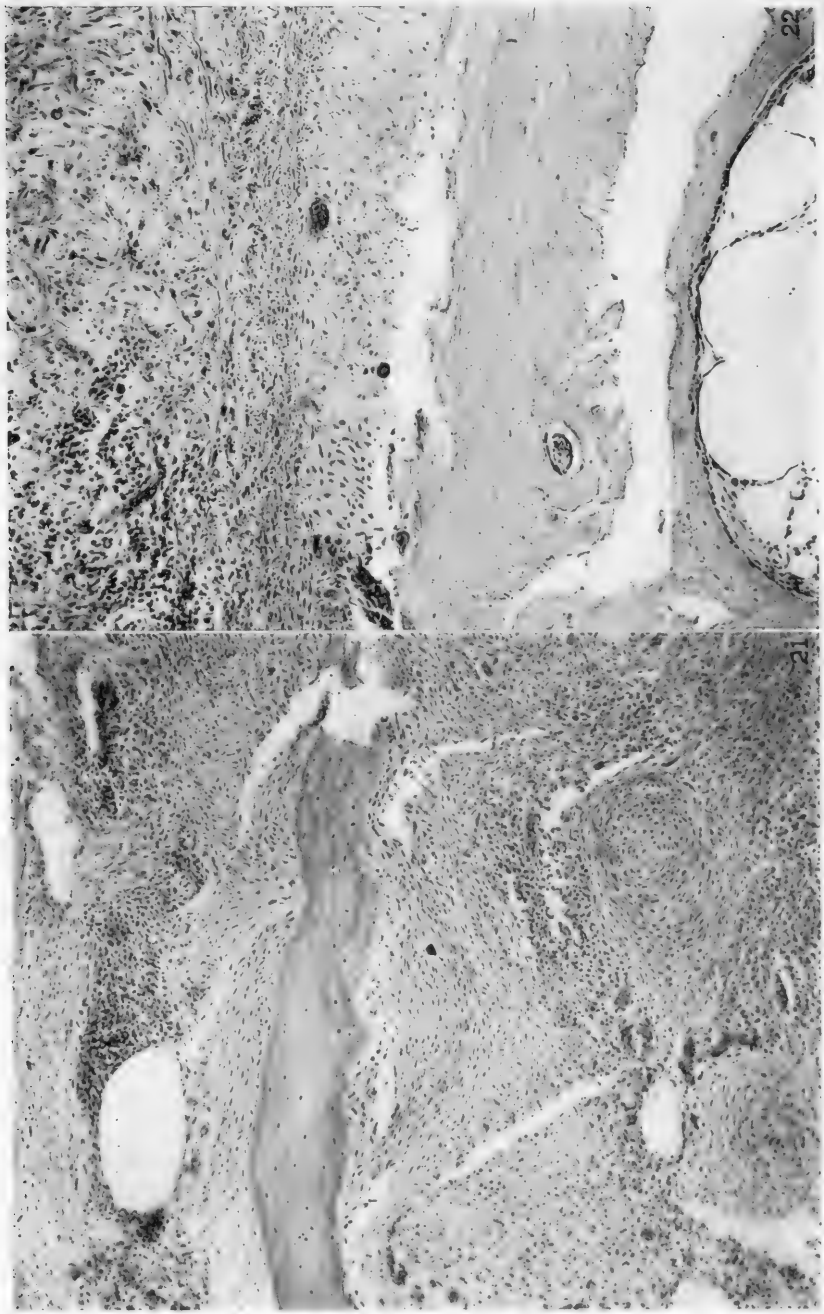


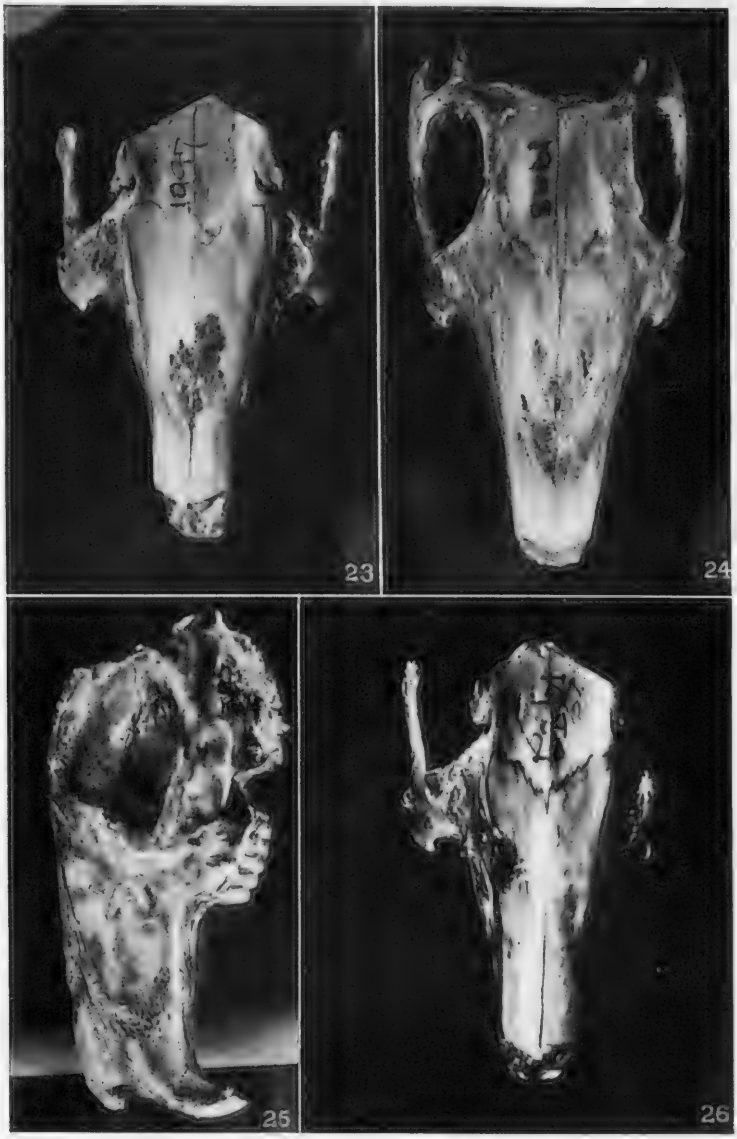
(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)



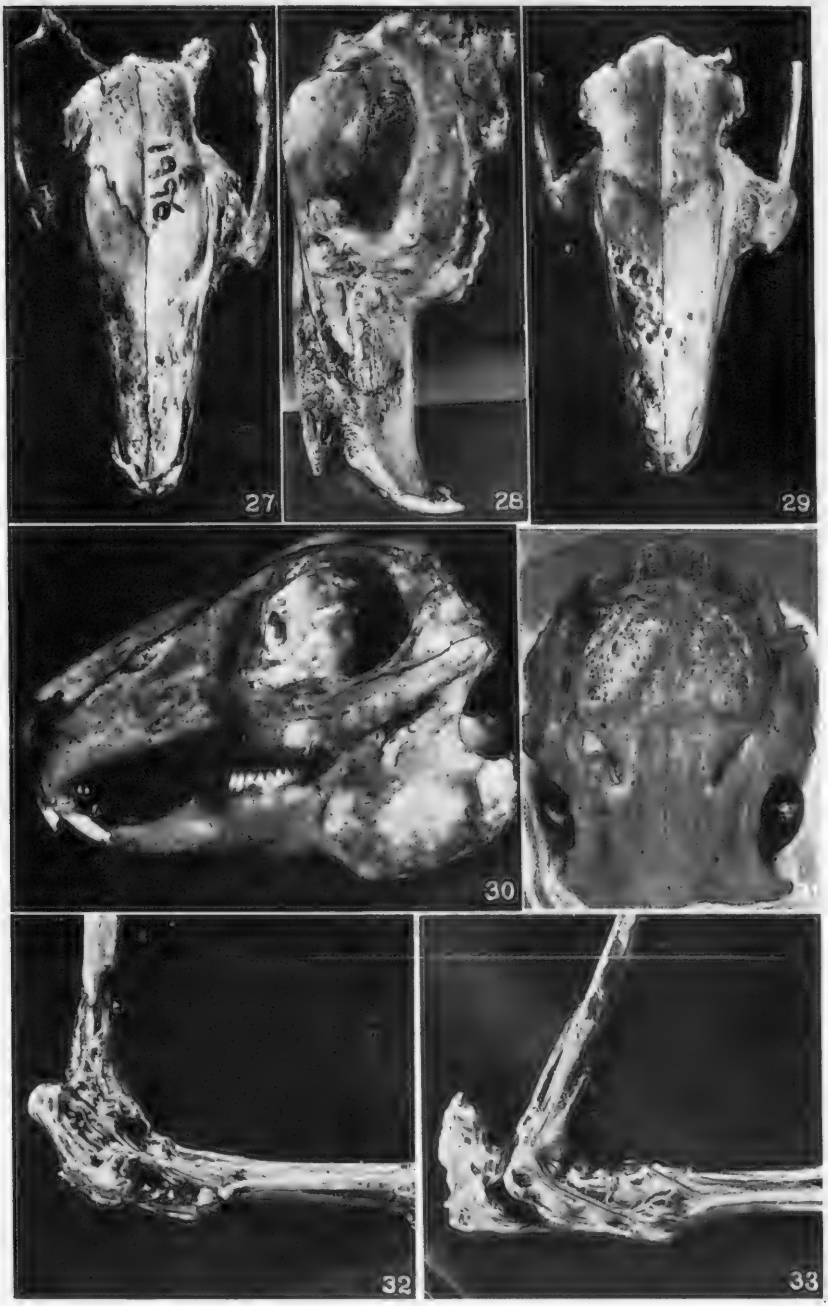
(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)



(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)



(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)



(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)

EXPERIMENTAL SYPHILIS IN THE RABBIT.

VI. Affections of Bone, Cartilage, Tendons, and Synovial Membranes.

Part 2. Clinical Aspects of Syphilis of the Skeletal System. Affections of the Facial and Cranial Bones and the Bones of the Forearm.

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Plates 61 to 66.

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Syphilitic affections of the skeletal system were described in the preceding paper (1) from the standpoint of the pathological process, and it is the purpose of this paper to correlate these changes with clinical manifestations of the disease. This phase of experimental syphilis is of unusual importance on account of the widespread and frequently obscure nature of the infection. The subject will be dealt with, therefore, in somewhat greater detail than other groups of affections with the belief that the facts presented have an important bearing upon latent or obscure infections in the human subject and especially upon congenital forms of the disease.

The conditions to be described are numerous and it will be necessary to divide the material into two papers, the first of which will deal with affections of the facial and cranial bones and the bones of the forearm. The remaining subjects will be considered in the second paper.

Parts Involved and Character of the Affection.

The most obvious affections of the skeletal system were those of the face and of the feet and legs, but lesions were also found in many other parts of the body, such as the cranial bones, the cervical and

caudal vertebræ, the ribs, and the sternum. The proportion of focal infections which gave rise to clinical signs of disease cannot be estimated, but it seems probable that many obscure infections occurred which did not give rise to any obvious abnormality.

Bone infection was recognized in general by the development of visible or palpable enlargements over the surface of the bones, or, in the absence of these, by rarefication and the loss of structural detail as indicated by the radiograph, and eventually by necrosis and disintegration of the bone. The skin over the lesion was freely movable, while the nodular mass was firmly attached to the underlying bone. In rare instances, the skin might become adherent, but it is worthy of note that necrosis and ulceration of the skin were never observed in connection with lesions of either tendons or bones, with the exception of the phalanges. The diagnosis of these conditions as syphilitic affections was readily made by the demonstration of spirochetes in the lesions.

These were the general characteristics presented by all classes of bone infections, but the details of the processes as they occurred in different parts of the body were subject to considerable variation.

Facial and Cranial Bones and Cartilages.

Judged upon the basis of the occurrence of lesions which were sufficiently pronounced to be detected by ordinary methods of examination, the facial bones were more often the seat of syphilitic affections than those of any other part of the body, with the possible exception of the hind feet and legs. The bones usually involved were the nasals, the two lateral splints composed of the frontal process of the premaxilla and the maxillary process of the frontal, the turbinates, and to a lesser extent the maxillæ, the premaxilla, and the frontal eminences. These bones lie immediately around the nasomaxillary fossa, and, with few exceptions, the lesions of the facial area were confined to the region of this fossa and the bridge and sides of the nose. The most frequent locations were the bridge of the nose, the end of the nose, and the bony ridge extending from the naso-orbital angle along the sides of the nose, while young animals in particular showed a marked tendency to localization at the point of union of the frontal and maxillary processes.

The appearance presented by animals with well marked facial lesions of various types is shown in Figs. 1 to 7, 11, and 15. Fig. 1 represents a very common type of deformity due to a sharply circumscribed periosteal lesion on the bridge of the nose. Fig. 2 is a more pronounced condition of a similar character which involved the lower two-thirds of the nasal bones and the lateral splints and extended well down on the premaxilla.[1] The profile of the animal shows a gradual curve extending from just below the level of the eyes to the tip of the nose, the deformity being less abrupt than that in the preceding case, and hence not so noticeable at first.

A less common affection is that illustrated in Fig. 3. This was a multinodular lesion of the lower portion of the nasal bones and cartilages and was the most pronounced case of its kind which occurred in our series of animals, measuring 1.85 by 1.7 by 1.2 cm.

Another unusual type which was associated with a marked alteration of the profile is that shown in Fig. 4. The prominence seen above the eye of this animal was due to a large periosteal granuloma involving the supraorbital portion of the frontal bone. This process was bilateral.

The visible deformity in all these animals was quite apparent, but there were many instances in which little or no visible alteration in contour could be detected either on account of the size and form of the lesion or its location. This was especially true when the sides of the nose and the region of the nasomaxillary fossa were involved. Photographic reproduction of even large lesions in these locations was found to be extremely difficult. This class of affections is illustrated by two animals (Figs. 5 and 6) both of which represent very common types of facial involvement.

The lesions of the animal in Fig. 5 consisted of a multinodular mass which extended downward along the bony ridge at the sides of the nose from the region of the naso-orbital angle and then across the bridge of the nose. They were quite large and yet very little of the lateral deformity could be seen until the hair was clipped, disclosing the marked increase in breadth or fullness of the midnasal region which is fairly well shown in the photograph.

In Fig. 6, there is a large, flat, and slightly oval lesion covering the upper half of the right nasal bone and extending well back over the nasomaxillary fossa. It measured more than 0.5 cm. at its center (determined after resection), and yet no deformity of the face was apparent until the hair was removed.

In like manner, the symmetrical deformity produced by many bilateral lesions was even less evident than that of unilateral affections,

[1] See Brown, Pearce, and Witherbee (1), Fig. 28.

especially in the naso-orbital angles or along the upper portions of the nasal splints, which are frequently involved. The appearance presented in the majority of these cases was merely that of a fullness between the eyes or of a slight prominence in the naso-orbital curve.

Facial abnormalities were more apparent with periosteal lesions arising from the outer surface of the bones than with other forms of bone involvement, unless the lesions extended to the outer surface, in which case they frequently assumed an appearance similar to the conditions described.

The extent of the process and the damage produced by infections of the facial bones bore no particular relation to the size of the external growth. These changes could be gauged by palpation, by sounding the lesion with a pointed instrument, or by radiographs. The information to be derived from the use of the latter method of investigation may be illustrated by a series of animals in which the appearance of the lesion, the radiograph, and the bone specimen are compared (Figs. 7 to 16).

The first animal in this series had a marked diffuse periostitis of the nasal bones which produced a typical Roman nose deformity (Fig. 7). By palpation, it was found that the entire lower portion of the bone gave beneath the finger, and a radiograph taken at this time (Fig. 8) showed complete destruction of the mid-area of the nasal bones together with some clouding and loss of architecture in adjacent parts of the bone. After about 2 weeks, the lesion began to subside and regeneration of the bone could be detected. At the end of 6 weeks, a second radiograph (Fig. 9) showed a marked but irregular thickening of the nasal bones and an absence of the usual architecture. There was also a bony mass extending below the level of the nasal bones. The actual condition existing is shown by Fig. 10 which is a photograph of the dried skull.

A second, more significant case is illustrated in Figs. 11 to 14. This was an instance of diffuse periostitis which produced very little facial deformity. A slight thickening could be made out over the bridge and lower portion of the nose and a defect could be detected along the sides of the nasal bones near the end of the nose. The radiograph of these lesions showed very plainly that there was some bone destruction as indicated by the ragged appearance and the thinning out of the under side of the shadow near the end of the nose (*cf.* Fig. 13 which shows the normal appearance). The fine lines of shadow produced by the turbinates were also blurred, and there were clouding and loss of architecture throughout most of the nasal bone. When these findings were compared with the autopsy specimen (Fig. 14), it was found that the destruction was somewhat greater than might have been imagined from the radiograph.

The late effects following repair in a similar case of diffuse periostitis are shown in Figs. 15 and 16. In this animal, there was a permanent enlargement of the end of the nose (Fig. 15) due in part to a fibrous thickening of the periosteum and in part to a thickening of the nasal bones as shown in Fig. 16. The condition had existed for upwards of 2 years.

Infections arising from the interior of the nose were characterized clinically by one or more of three conditions: the presence of a nasal discharge containing spirochetes, necrosis of the outer covering of bone, and finally, the development of external granulomatous lesions. Few of these infections have been recognized clinically and our knowledge of them is mostly that of autopsy findings. Involvement of both the nasals and the turbinates has been demonstrated in this way, but it will be seen at once that in the absence of any external lesion, there is no simple method of recognizing affections of this class or of differentiating them from affections of the mucous membranes. It is not improbable, therefore, that many internal lesions may escape detection and that many instances of nasal discharges containing spirochetes and of obstructive phenomena of the nasal passages are referable to bone infection rather than to primary infection of the mucous membranes. This supposition is supported by abundant pathological evidence.

The probability of the occurrence of lesions other than those described must not be overlooked. Several instances of peculiar thickening of the frontal and parietal bones of rabbits with generalized syphilis have been noted, and there was one animal with a marked mandibular affection. These lesions were all inactive, and there was nothing present which would enable one to determine their cause with certainty. However, a case of necrosis of an occipital condyle was observed, in which the infection was still active. The circumstances in this case will be given later (2).[2]

The points of especial interest in connection with syphilis of the facial bones are the frequency, the location, and the destructiveness of the lesions. It is also important to note the close analogy which exists between certain of the nasal and supraorbital affections of the experimental animal and those of man, especially in the congenital form of the disease.

[2] Brown, Pearce, and Witherbee (2), p. 531.

Forearms.

Bone affections of the anterior extremities of the rabbit were, with one exception, represented by a periostitis involving the distal ends of the ulna and radius. The majority were situated on the extensor surfaces at or near the epiphyseal line, with an occasional lesion at a slightly higher level.

This type was of comparatively common occurrence. As a rule, the lesions were rather small and with the fur intact produced little or no alteration in the appearance of the affected part but were easily detected by palpation. Normally, the extensor surface of the forearm is either perfectly smooth or is marked by small cross ridges and angular projections at the epiphyseal lines and the heads of the radius and ulna respectively. When lesions develop at these points, oval or rounded nodules are formed which are very readily felt on examination and can be seen after removal of the hair.

A typical case of bilateral involvement of both ulna and radius is shown in Fig. 17 (*cf.* Fig. 18 in which the left forearm is normal). Three of the four nodes present were distinctly rounded, while that on the right radius was more oval and covered a greater area of the bone.

A second type of affection which was frequently encountered is that seen in Fig. 18. In this instance, there was only one lesion, which formed a rather large oval mass on the right ulna just above the carpus.

In exceptional instances, the lesions of these bones were much more marked than those described and produced very striking deformities, as in Figs. 19 and 20. This, again, was a bilateral affection of both the ulna and the radius. As may be seen from Fig. 20, the lesion of the ulna was very sharply demarcated and raised abruptly from the surface of the bone, while that of the radius was more diffuse and resulted in extensive necrosis of the distal end of the bone.

A second case of an even more marked character is illustrated in Figs. 21 and 22. This was a large oval lesion which arose from the ulna but extended to the radius by way of the interosseous membrane. It was one of the few instances in which definite necrosis of the bones of the forearm could be made out clinically. As shown in the accompanying radiograph (Fig. 22), there was necrosis of the lower end of the ulna as well as the contiguous margins of both the ulna and the radius. The lesion was of very rapid growth, reaching the stage shown within 9 days after it first appeared. The tissues surrounding it were of a violet-red color, and there was a pronounced edema of both the skin and subcutaneous tissues.

In addition to the affections described, mention may be made of an enlargement of the carpus, which was followed by a permanent deformity occurring among the first animals of our series, but the methods then in use were not sufficiently developed to enable us to determine the nature of this condition. From the circumstances, it seems probable, however, that this might have been due to syphilitic infection. No other case of the kind has come under our observation.

The examples of periostitis of the ulna and radius which have been cited were cases in which there was not more than one lesion on each bone. Multiple lesions of one or both bones were occasionally seen, but they were usually small. In many instances, only one forearm was affected, but more often the involvement was bilateral and symmetrical.

It was a notable feature of this group of lesions that they rarely led to extensive necrosis. Grossly, the surface of the bone was eroded or roughened, and obliteration or widening of the epiphyseal line could be demonstrated by use of x-rays. Similarly, when the lesion healed, the bone might show a slight roughening or increase in thickness with a few tiny nodes (see lateral margin of the ulna in Fig. 22), but rarely was there any considerable deviation from the normal. Microscopic examination showed, however, that the syphilitic process frequently extended through the entire thickness of the bone and that the effect produced was in general much greater than gross appearances would indicate.

Other aspects of the subject of syphilis of the skeletal system will be presented in Part 3 of this paper (2).

BIBLIOGRAPHY.

1. Brown, W. H., Pearce, L., and Witherbee, W. D., *J. Exp. Med.*, 1921, xxxiii, 495.

2. Brown, W. H., Pearce, L., and Witherbee, W. D., *J. Exp. Med.*, 1921, xxxiii, 525.

EXPLANATION OF PLATES.

All illustrations are reproductions of photographs or radiographs which have not been retouched and represent objects at their natural size. Statements of time are estimated from the date of inoculation unless otherwise indicated.

FIGS. 1 to 6. Appearance presented by animals with syphilitic affections of the facial and cranial bones.

PLATE 61.

FIG. 1. 146 days. Nodular periostitis, bridge of the nose.

FIG. 2. 105 days. Marked diffuse periostitis of the lower two-thirds of the nasal bones.[1]

FIG. 3. 159 days. Marked nodular periostitis and perichondritis of the nasal bones and cartilages.

FIG. 4. 71 days. Marked periostitis of the supraorbital region.

PLATE 62.

FIG. 5. 110 days. Multinodular periostitis of the nasomaxillary fossa, the sides and bridge of the nose. The lesions were very pronounced.

FIG. 6. 92 days. Periostitis of the right nasal bone and splint. The lesion formed a broad flattened mass which covered the upper half of the bone and adjacent portions of the fossa.

FIGS. 7 to 16. A comparative study of clinical and pathological conditions in syphilitic affections of the nasal region.

PLATE 63.

FIGS. 7 to 10. Marked diffuse periostitis of the nasal bones and splints with necrosis and regeneration.

FIG. 7. 68 days. The deformity of the nose. The lesion was centered over the bridge of the nose and extended from just below the level of the eyes to the tip of the nose.

FIG. 8. 68 days. Radiograph showing complete necrosis of the bone at the center of the process. The turbinates are also involved.

FIG. 9. 6 weeks later. Regeneration of bone. The nasal bones are considerably thickened, irregular, and devoid of architecture. There is also an irregular osteoid mass beneath the nasal plates.

FIG. 10. 121 days. Lateral view of the skull showing the regenerated bones.[3]

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[3] For frontal view see Brown, Pearce, and Witherbee (1), Fig. 29.

PLATE 64.

FIGS. 11, 12, and 14. Diffuse periostitis which produced very little alteration in the facial contour.

FIG. 11. 102 days. A slight prominence towards the end of the nose was the only visible abnormality. Palpation showed necrosis of the margins of the nasal bones.

FIG. 12. 116 days. Radiograph showing clouding of the nasal bones and erosion of the under side near the end of the nose. There are also a marked clouding and loss of finer shadow details throughout the nasal chambers and parts of the maxilla and premaxilla, indicating an extensive involvement of these parts.

FIG. 13. Normal radiograph for comparison.

FIG. 14. 123 days. Skull of the animal in Figs. 11 and 12.[4]

PLATE 65.

FIGS. 15 and 16. 2 years and 2½ months. Chronic fibrous periostitis with bulbous thickening of the end of the nose—a permanent condition. The enlargement of the nose was due in part to fibrous tissue and in part to thickening of the bone. In the radiograph (Fig. 16) note the change in shape and prolongation of the nasal bones, eburnation, and increase in osteoid tissue on the under side of the nasal bones and throughout the nasal region.[5]

FIGS. 17 to 22. Syphilitic affections of the radius and ulna.

FIG. 17. 61 days. A typical bilateral periostitis of the radius and ulna.

FIG. 18. 92 days. A typical case of unilateral periostitis of the ulna (right) with extension to the interosseous membrane and the adjacent margin of the radius. The left forearm is normal.

PLATE 66.

FIG. 19. 76 days. An unusually marked affection of both the ulna and radius. The condition was bilateral.

FIG. 20. 77 days. Autopsy specimen of the same lesions. That of the ulna is seen to be a sharply demarcated periosteal granuloma, while that of the radius is an osteitis of the head of the bone.

FIG. 21. 60 days. Marked periostitis and osteitis of the distal ends of the ulna and radius.

FIG. 22. 61 days. Radiograph of the same lesions. Note the irregularities and rarefication of the lower ends of the bone and the fusiform enlargement of the radius.

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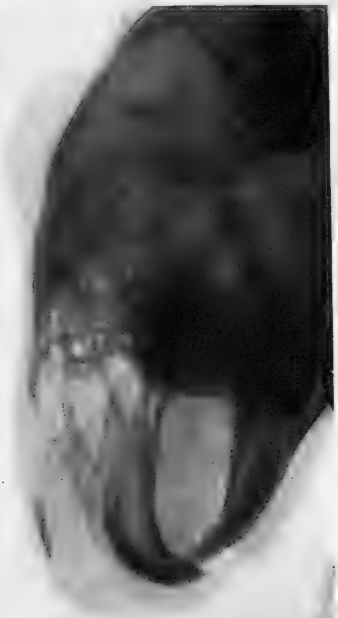
[4] For frontal view see Brown, Pearce, and Witherbee (1), Fig. 27.

[5] For skull see Brown, Pearce, and Witherbee (1), Figs. 6 and 7.

(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)

(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)



(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)

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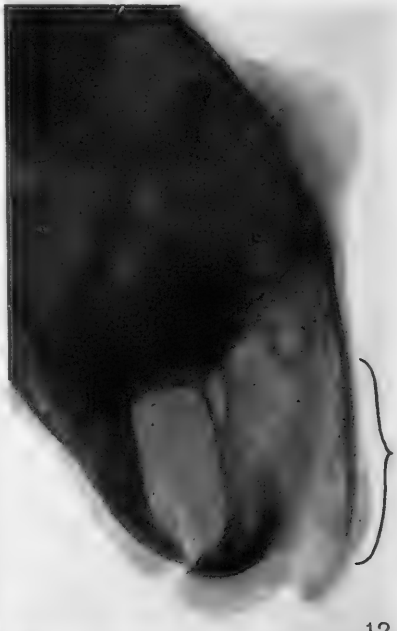

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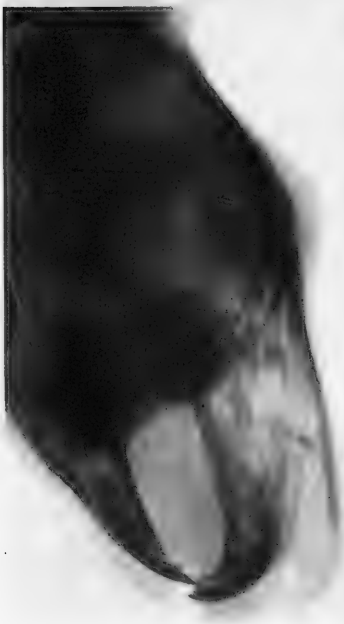

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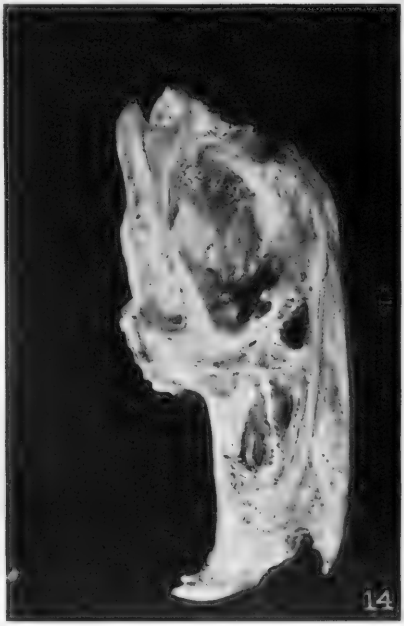

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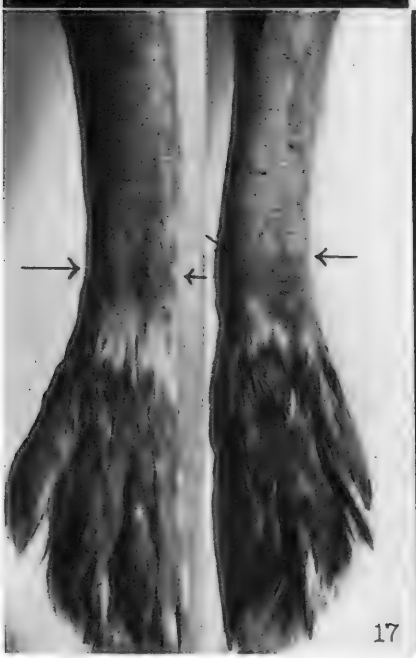
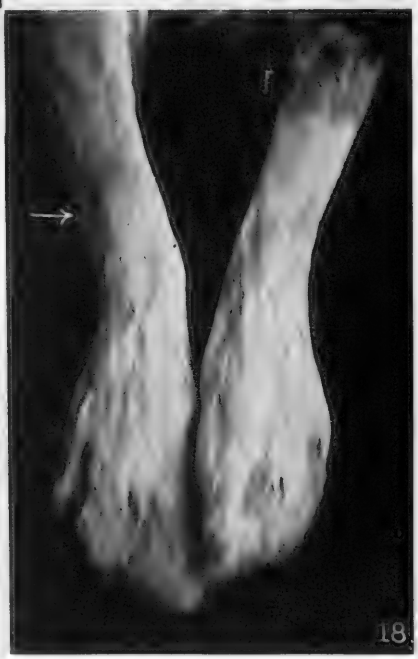
(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)





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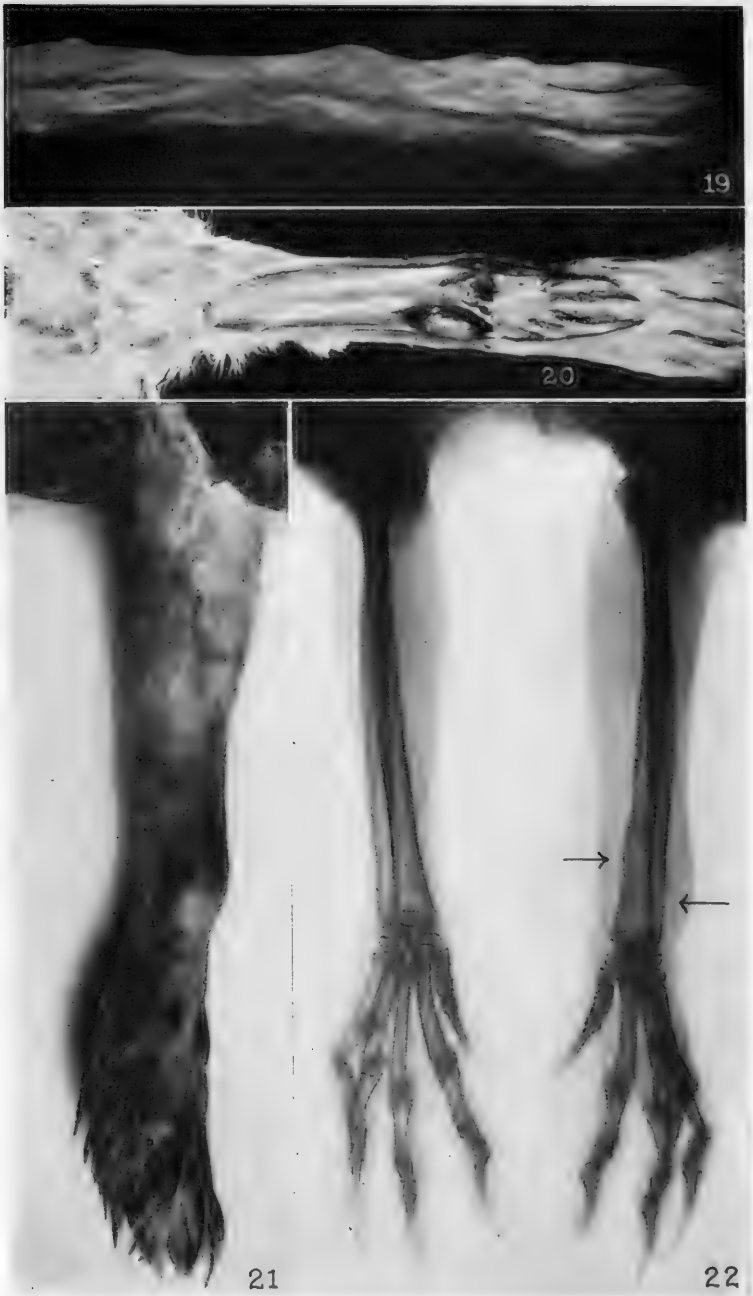

(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)

EXPERIMENTAL SYPHILIS IN THE RABBIT.

VI. Affections of Bone, Cartilage, Tendons, and Synovial Membranes.

Part 3. Syphilis of the Posterior Extremities with Other Affections of a Miscellaneous Type.

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Plates 67 to 72.

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The phases of syphilis of the skeletal and supporting systems to be considered in this paper are the affections of the hind feet and legs, and of the vertebral column, joint cavities, and tendons with the clinical history of this form of experimental syphilis.

Hind Feet and Legs.

In our first series of animals, there were fewer instances of involvement of the bones of the hind feet and legs than of the forearm, which was the reverse of the condition noted with the cutaneous lesions. Among the more recent cases of generalized syphilis, however, the incidence of bone lesions of the posterior extremities was markedly increased, and the lesions themselves were much more pronounced. The bones involved were the lower end of the tibiofibular, the tarsals, the metatarsals, and the phalanges. The affections included both periosteal and endosteal processes with a considerable number of cases resulting in epiphyseal separation or pathological fracture.

Tibiofibular.—The most easily recognizable lesions of the lower extremities were those of the tibiofibular, all of which were periosteal in origin and with a single exception occupied essentially the same position; namely, the anterolateral surface of the lower end of the bone, or what might be termed the external malleolus. This position

corresponds to the line of union between the tibia and the fibula and normally is marked by a small depression surrounded by a series of angular points or ridges. The development of a periosteal lesion in this location caused a filling in of the depression and rounding off of the bony prominences.

Affections of this type were quite common. Some of the lesions were of a comparatively small size, while others reached a centimeter or more in diameter and produced a very characteristic deformity, as may be seen by reference to Figs. 1 to 3.

In this animal, there was a bilateral involvement of the bones, the lesions of the two sides being of approximately the same size and character. The right leg (Fig. 1) illustrates the appearance presented with the fur intact, while the left, with the hair removed, shows more accurately the location of the lesion and the actual deformity produced. The autopsy preparation in Fig. 2 shows the appearance with the skin reflected.

None of the affections of this group appeared to produce any considerable gross alteration in the bone. The changes noted were analogous to those of the ulna and radius. Fig. 3, which is a radiograph of the lesions in Fig. 1, shows the condition usually found. On the right, there is a very definite thinning of the outer table and some irregularity in the surface of the bone. The surface of the left tibia is less affected and shows no more than a suggestion of a roughening, but there appears to be some rarefication in the lower end of the bone, and the most marked alterations were probably in the heads of the bones. This radiograph may also be used as a means of orientation for the lesions and the parts of the bone affected.

Minor variations in the location of the lesions were not uncommon, but in only one instance was the position materially different from that described. In addition to the usual involvement of the external malleolus, this animal developed a third lesion which took the form of an annular periostitis surrounding the shaft of the right tibiofibular over an area about 1 cm. in breadth and at a level of 2 to 3 cm. above the distal end of the bone.

Metatarsals.—While affection of the metatarsals was of frequent occurrence, the lesions were, with few exceptions, confined to the so called fifth or lateral metatarsal; the other bones involved were the first and the fourth. This group of affections presented a variety of conditions consisting chiefly of periosteal lesions but including also affections of the bone and a considerable number of cases of epiphyseal

separation. While some were small and rather difficult to detect, except by the most careful palpation, the usual affection was quite obvious to one familiar with the topography of this part of the foot.

The fifth metatarsal of the rabbit is much the same as that of man. The features of especial importance are the presence of a sharp bony prominence, or hook, formed by the tuberosity at the proximal end of the bone and the narrow tendinous ridge which extends along the lateral margin of the foot throughout the length of the bone. These features of the normal foot are shown in Fig. 4.

The tuberosity, or proximal end, of the metatarsal was perhaps most frequently affected. Irrespective of its origin, the condition usually produced was of the nature of a firm swelling which centered about the head of the bone and extended some distance along the adjacent portion of the shaft. The picture presented by such affections was that shown in Fig. 5. The bone injury produced in this case is shown in Fig. 6 which is a radiograph of the feet in Figs. 4 and 5.

Occasionally, much greater proportions and a more destructive character were noted; an example of this kind is given in Figs. 7 and 8. As in the preceding case, the lesion arose from the head of the fifth metatarsal but extended to the adjacent portions of the fourth metatarsal (Fig. 8); there was practically complete necrosis of the head of the fifth metatarsal followed by the formation of an irregular bony mass which is well shown in the radiograph (Fig. 8).[1]

A second group of lesions occurred along the shaft of the bone either singly or as multiple nodules. The majority of these were periosteal affections; they were usually located on the lateral or dorsal surface but were occasionally found on the plantar surface or completely encircled the bone. The usual form was that of a fusiform swelling such as is shown in Fig. 9. A more pronounced case of a similar character is seen in Fig. 10. In this instance, there were two distinct lesions present, one of which appeared as a spherical mass embracing the head of the metatarsal and the other as a fusiform enlargement extending the full length of the shaft. The condition was bilateral and symmetrical.

[1] Fig. 16 is taken from the same animal.

None of the periosteal lesions of the shaft thus far examined have resulted in a gross alteration which amounted to more than a slight erosion of the bone or slight roughening and thickening which persisted for some time after the healing of the lesion (see Fig. 21). Microscopic changes, however, were more marked.

In a few instances, affections of the bone or medullary cavity were discovered by x-ray examinations and verified by autopsy, but very little is known of their clinical history. At times, they produced conditions analogous to those of periostitis or resulted in necrosis and pathological fracture.

A third group of lesions arose from the distal end of the metatarsal, and while many of these were simple periosteal processes analogous to those of the shaft and the proximal end of the bone, they were of peculiar interest on account of the frequent occurrence of pathological fractures or epiphyseal separations. At present, we cannot attempt to differentiate between the various types of pathological processes which were concerned in the production of these conditions. Some were obviously the result of periosteal infections, while others appeared to be due to infection within the bone itself or to a combination of the two processes. An example of each of these may be given.

The lesion which is illustrated in Figs. 11 to 13 began as a simple periosteal process, and the first radiograph showed little if any alteration in the bone. Growth was unusually active, however, and within a week, slight crepitus could be detected and the radiograph showed the presence of a fracture of the neck just proximal to the epiphyseal line (Fig. 13). It could not be determined whether this was due entirely to the lesion of the periosteum or to the presence of a second focus of infection within the bone which was obscured by the periosteal lesion. When the animal was killed 11 days later, the bone was found to be irregularly and markedly thickened in its outer third.

The second case, illustrated in Figs. 14 and 15, is what appears to be a clear instance of an epiphyseal infection, or an osteochondritis, and is of especial interest on account of its occurrence in an adult animal. When first noted, there was a firm narrow ridge at the epiphyseal line which was barely palpable and could not be diagnosed with certainty as a syphilitic lesion. This ridge increased slightly, and within a few days, an edematous swelling appeared, the extent of which is indicated by the shadows in the radiograph taken at this time (Fig. 14). There was a distinct crepitus, and the radiograph showed an obliteration of the epiphyseal line with a clean-cut separation of the epiphysis (Fig. 14). The lesions healed with a slight bony thickening, and it will be noted that the epiphyseal line was not reestablished (Fig. 15).

From the description of metatarsal lesions which has been given, it will be seen that the essential differences between affections of these bones and those of the tibiofibular or ulna and radius were the extent of the bone involvement and the occurrence of focal infections within the bone and especially at lines of epiphyseal union.

Tarsus.—The status of syphilitic affections involving the tarsus is still somewhat obscure. As was pointed out in Part 1 of this paper (1), there is a variety of conditions affecting this part of the body which present much the same clinical picture. Those observed were chiefly affections of the calcaneus and included a few cases of syphilitic periostitis with numerous instances of necrosis of the calcaneus, pathological fracture, and epiphyseal separation together with some instances of extension of the infection to other bones of the tarsus or to ligaments, tendons, and synovial membranes.

All the periosteal lesions of the calcaneus were situated on the dorsal or superior surface of the bone near the anterior end. Normally, there is a well marked depression at this point which, in cases of periostitis, was obliterated or filled by a firm elastic mass. The resulting alteration in appearance is shown in Figs. 11 and 12, which may be compared with other illustrations.

So far as we are aware, in no instance was any marked necrosis of the underlying bone produced, but it is important to note that the majority of the cases of necrosis of the calcaneus which occurred in the absence of any known lesion of the periosteum occupied exactly the same position. This is clearly illustrated in Fig. 16.

The conditions designated as necrosis of the calcaneus, pathological fracture, and separation of the epiphysis have been considered in detail elsewhere (1). It seems necessary only to recall that all presented a common symptom-complex, the outstanding features of which were the sudden development of lameness, an edematous swelling of the tarsus, tenderness, and crepitus, the cause for which could be determined with accuracy only by radiograph or by careful dissection. The swelling and edema are well shown in Fig. 16.

In a few instances, other bones than the calcaneus have been found to be involved in affections of the type described and as a result of extension of lesions from such points as the head of the metatarsals (Fig. 16). However, they presented no distinctive clinical characteristics.

Phalanges.—As one passes from the larger and more accessible bony structures to those which are extremely small or difficult of examination, the question of the presence or absence of bone lesions and the nature of the injury become less certain. This applies to syphilitic affections of the phalanges and the bones of the carpus and tarsus as well. However, numerous instances of periostitis of the phalanges, or dactylitis, and of necrosis of the bones with no demonstrable lesion of the periosteum have been observed in the toes of the hind feet.

The occurrence of affections of the terminal phalanges which gave rise to abnormalities of the nails was referred to in connection with the cutaneous manifestations of syphilis (2, 3).

Lesions of this type are shown in Figs. 17 to 19 and also on the fifth toe in Fig. 8. There is a periostitis of the terminal phalanges of several of the toes in Figs. 17 and 18, and the loss of the nail can be traced in the fourth toe of the left foot. Fig. 19 shows a more extreme condition, which was permanent. Enlargement and induration of the terminal phalanx, as in these cases, rarely occurred without the production of an easily recognizable paronychia.

Recorded instances of a simple periostitis of the phalanges which did not involve the skin or its appendages were comparatively few. However, a large proportion of these cases occurred on the fifth or lateral toe as in Figs. 20 and 21, thus conforming to the order of distribution recorded for other lesions of the hind feet. In this connection, attention may be called to a striking tendency towards the occurrence of serial periosteal lesions along the outer sides of the feet, producing a beaded effect which is well shown in Fig. 21.

Lesions were observed on the first, second, and third phalanx; some produced no appreciable alteration in the bone, while others resulted in necrosis which was occasionally followed by fracture or disintegration.

Infections arising within the bone were discovered in a few animals, and their development was followed by serial radiographs. There were no appreciable external evidences of bone involvement in these cases until necrosis and disintegration or fracture of the bone occurred. The parts then became more or less swollen and tender, and in some instances a crepitus could be detected.

Several examples of affections of this kind may be seen in Figs. 16 to 18. The lesion in the first phalanx of the outer toe of the right foot in Figs. 17 and 18 is brought out especially well. By careful inspection (use reading glass), it will be seen that there is an enlargement of the distal end of this phalanx (Fig. 17) with rarefication and loss of architecture, but that there is no shadow to indicate the presence of a periosteal lesion. 13 days later (Fig. 18), a fracture occurred at this point accompanied by the usual reaction in the surrounding tissues.

There were a number of definite bone lesions present in the feet of this animal, none of which could have been clearly recognized except by radiographic examination; there were periosteal lesions of several toes (outwardly paronychias), and osteitis in the phalanx mentioned and at both extremities of the fifth metatarsal of the same foot. In Fig. 16, a number of the phalanges are also seen to be involved. This will serve to indicate the difficulty in the recognition of lesions of this type, our knowledge of which is at present rather limited.

Obscure Bone Affections.

The conditions which have been described in the preceding pages may with reason be regarded as the obvious bone affections in contradistinction to infections which are accompanied by no signs which are sufficiently distinctive to enable one to recognize them clinically or even to suggest the possibility of their existence. It is quite certain that such conditions do occur as indicated by the frequency with which affections of the interior of the nares and of the small bones of the feet have been discovered by radiographic or pathological examination.

Other positions in which we have reason to believe that obscure affections may exist are the vertebral column, the cranial bones, the sternum, the ribs, and the mandibles. In each of these sites, lesions have been found whose pathology was sufficient to identify them as syphilitic. Infection of the caudal vertebræ has been recognized clinically in several animals, the condition being manifested as fusiform swellings. In other instances where no injury was suspected, necrosis of the bones was demonstrated by radiograph.

Our attention was attracted to two cases of infection of the cervical vertebræ by the development of symptoms of meningitis. In one of these animals, postmortem examination revealed the presence of necrosis of the cervical vertebræ, and spirochetes were demonstrated in the cerebrospinal fluid. In the other, the upper cervical vertebræ

and one of the occipital condyles were involved. Histologically, these bones showed a typical syphilitic reaction in the Haversian canals and marrow spaces, with absorption and necrosis of bone.

In two instances, lesions were discovered at autopsy at the costochondral junction of one of the ribs which corresponded grossly and histologically with other bone lesions in the animals. In another animal, a healed lesion was found in the xiphoid and the adjacent part of the body of the sternum which appeared to be of syphilitic origin, and a similar condition was observed in the mandible of another animal.

The occurrence of obscure bone affections and infections associated with very slight local reaction is of considerable importance and lends support to the belief that many cases of latent or obscure human infection may find their explanation in the occurrence of an analogous group of conditions.

Tendons and Synovial Membranes.

In Part 1 of this paper (1), mention was made of the occurrence of affections of tendons, joints, and synovial membranes. With the exception of the tendons, these conditions were not accompanied by signs which were sufficiently distinctive to permit their clinical recognition, and nothing can be said of their clinical history.

Only a few cases of primary tendon involvement were recognized clinically, all of which occurred in the tendo achillis. The lesions were usually small nodular, or fusiform masses which could be detected only by palpation. In one instance, however, the lesion developed on the outer side of the tendon and produced a visible enlargement over which the skin was freely movable.

Clinical History of Bone Affections.

The clinical history of bone affections cannot be given in so complete form as one would wish, since only a few animals of this group were held for any considerable period of time; our knowledge is, therefore, chiefly of the early stages of the infection.

From available data, it would appear that syphilis of the bones occupies a position with relation to the infection as a whole which is closely analogous to that of the cutaneous manifestations.

Among conditions which seemed to exercise a predisposing influence upon the occurrence of bone infection, mention may be made of the reduction or early suppression of primary lesions by unilateral instead of bilateral inoculation, by castration or excision of lesions, or by the use of therapeutic agents. When procedures of this kind were employed, the relative incidence as well as the extent of the bone involvement was definitely increased. The peculiarities of distribution and the marked predilection for unprotected bony prominences would indicate that the factor of trauma also plays a part both in the occurrence and in the localization of this class of lesions.

The important point to be noted, however, is that lesions of periosteum and bone appear to occupy a definite position in the scheme of defensive reactions in the experimental animal. Initially, this reaction is bound up with the development of the primary lesions, but if the latter are suppressed at an early stage of the infection, it is almost invariably taken up by the periosteum and bone. Conversely, if the primary lesions are allowed to progress uninterruptedly, or if they are suppressed late in the course of the infection, protection is apt to be conferred upon this group of tissues, and the burden of reaction is assumed by some other set of tissues such as the skin or the mucous membranes. The detailed consideration of the facts upon which this statement is based will be taken up elsewhere (4).

Time of Occurrence.—From what has been said, it may be surmised that the time of appearance of bone lesions varied greatly. Under conditions which favored their development, they were among the earliest manifestations of a generalized disease. While the extreme limits of variation in our series of animals were from a minimum of 38 days to $15\frac{1}{2}$ months after inoculation, most often the lesions appeared within 2 to 3 months. The reactive period of bone infection, if we may term it such, was shorter and more sharply demarcated than that of other lesions. This was especially true in animals whose primary lesions had been suppressed. In these, the lesions appeared with great regularity at or near the expiration of the 60 day period following inoculation, those of the nasal region as a rule being recognized slightly earlier than those of other bones.

Course and Duration.—The course of the bone lesions was one of comparatively rapid development and rapid decline, in which there

was less of the cyclic variation to be seen than in almost any other class of syphilitic affections. Clearly defined periods of growth and regression were observed in some animals, but it is worthy of note that no instance of true relapse or recurrence of a healed lesion was recorded. In a single instance, in which a periosteal lesion on the nose of an animal persisted for more than a year, there were several periods of marked regression followed by renewed activity; this was the nearest approach to true relapse which was observed. Whether these peculiarities of bone lesions may be attributed to difficulties of observation on the one hand and, in our series, to the short period of observation on the other, or are characteristic of this group of affections cannot be said.

As a rule, the period of active infection was comparatively short. Some lesions disappeared within a few weeks, and few lasted for more than 1 to 2 months. The process of repair in cases of marked bone destruction was obviously more prolonged. In exceptional instances, active lesions persisted for several months; the longest recorded period in our series was 20 months, and this lesion was still active when the animal was killed.

Gross evidences of bone injury were, as a rule, comparatively slight and tended to disappear; in the case of the more destructive lesions, however, repair was less perfect and more or less permanent alterations were produced which were akin to those seen in syphilitic infections of man.

CONCLUSIONS.

The facts presented above indicate clearly that localized infections of periosteum, bone, cartilage, tendons, and synovial membranes form an important group of affections in rabbits inoculated with suitable strains of *Treponema pallidum*.

Among the more important features of this group, mention may be made of the early occurrence of the lesions and their relation to other manifestations of disease, the involvement of bones in exposed locations or of bones which are subject to pressure or trauma, the high incidence and destructiveness of affections of the nasal region, the tendency to localization at points of bony union or at epiphyseal lines, and the frequency of obscure focal lesions.

From a general standpoint, it will be seen that syphilis of the skeletal system of the rabbit is a very characteristic condition and that it combines features of both the acquired and the inherited form of the disease in man. It cannot be said that the two conditions are identical, yet they possess many fundamental characteristics in common, and it is believed that this form of experimental syphilis is of more than usual importance on account of the opportunity which it affords for the study of lesions of the skeletal system.

SUMMARY.

A systematic study was made of the affections of bone, cartilage, tendons, and synovial membranes which occurred in a series of rabbits with generalized syphilis. Localized infection of this group of structures was found to be of very frequent occurrence. The parts involved were, in the order of their frequency, the facial and cranial bones and cartilages, the bones, tendons, and joints of the feet and legs, the cervical and caudal vertebræ, the ribs, and the sternum. These infections often gave rise to characteristic manifestations of disease which could be detected without difficulty by inspection or palpation of the part. In many instances, however, clinical manifestations were so slight that the presence of lesions could be detected only by radiographic or pathological examination.

Detailed descriptions of various clinical types of disease were given and the clinical manifestations correlated with the pathological process. It was pointed out that bone lesions exhibited a decided predilection for certain exposed bony prominences, for lines of bony union, and for epiphyseal lines in particular.

A study of the clinical history of bone lesions brought out the fact that they were among the earliest of the generalized forms of disease; they tended to pursue a comparatively rapid course, and relapse was never observed.

Especial emphasis was laid upon three aspects of the experimental infection: the analogy existing between certain forms of the animal and human affections, the relation of syphilis of the osseous system to other evidences of disease, and the occurrence of obscure bone lesions.

In this connection, it was pointed out that the nasal and epiphyseal lesions of the rabbit presented a striking analogy to those of congenital syphilis in man.

It was also pointed out that syphilis of the osseous system occupied a definite position in the scheme of defensive reactions such that lesions of these tissues might be favored or inhibited according to the experimental conditions employed.

Finally, the frequency with which infections occurred which were not accompanied by sufficiently distinctive signs even to suggest the possibility of their existence was interpreted as evidence that some cases of latent or obscure infection in man might find their explanation in the presence of a similar group of affections.

BIBLIOGRAPHY.

1. Brown, W. H., Pearce, L., and Witherbee, W. D., *J. Exp. Med.*, 1921, xxxiii, 495.
2. Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1920, xxxii, 445.
3. Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1920, xxxii, 473.
4. Brown, W. H., and Pearce, L., *Arch. Dermatol. and Syphilol.*, 1921, iii, 254.

EXPLANATION OF PLATES.

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PLATE 67.

FIGS. 1 to 3. Syphilitic affections of the tibiofibular, or external malleolus.

FIG. 1. 84 days. A typical case of bilateral, nodular periostitis of the external malleolus.

FIG. 2. Autopsy specimen of the left leg showing the almost spherical granulomatous mass with some extension to the surrounding tissues.

FIG. 3. Radiograph showing location of the lesions in Fig. 1 with reference to bony parts and the apparent effect upon the underlying bone. Note the slight alteration in the margin of the shaft of the left tibia as compared with the right. The most marked changes are in the head of the bones (indicated by arrows). The lighter shadows in the left tibia are possibly due to lesions within the bone.

FIG. 4. Appearance of normal metatarsal.

FIGS. 5 to 15. Syphilitic affections of the metatarsals.

PLATE 68.

FIGS. 5 to 8. Lesions of the proximal end of the metatarsals.

FIG. 4. Lateral view of right hind foot showing normal topography of the metatarsal area.

FIG. 5. 135 days. Left foot of same animal showing a typical enlargement of the head and adjacent portions of the shaft of the fifth metatarsal due to syphilitic involvement of this portion of the bone.

FIG. 6. Radiographs of the feet shown in Figs. 4 and 5. Note the comparative condition of the proximal portions of the lateral metatarsals.

FIG. 7. 83 days. A marked syphilitic affection of the proximal ends of the fourth and fifth metatarsals.

FIG. 8. Radiograph showing the extent of the bone involvement.

PLATE 69.

FIGS. 9 to 13. Affections of the shaft and distal ends of the metatarsals.

FIG. 9. 117 days. A fusiform lesion of the shaft of the lateral metatarsal. Periostitis.

FIG. 10. 93 days. A marked periosteal affection of the entire fifth metatarsal. There is a nodular granulomatous lesion at the proximal end of the bone and a fusiform lesion extending its full length; this lesion completely surrounded the bone. There is also a skin lesion over the tarsus and just above this, a periosteal lesion of the tibiofibular.

FIGS. 11 and 12. 92 days. Dorsal and lateral views of a marked affection of the distal end of the fifth metatarsal. These photographs also show a prominent periosteal granuloma on the dorsal surface of the anterior end of the calcaneus (marked by arrow).

FIG. 13. Radiograph showing pathological fracture of the shaft of the metatarsal resulting from the lesion shown in Figs. 11 and 12.

PLATE 70.

FIGS. 14 and 15. Affections of the distal ends of the metatarsals.

FIG. 16. Affection of the tarsus.

FIG. 14. 58 days. An epiphyseal separation of the distal end of the fifth metatarsals. A displacement of the shafts and epiphyses is seen; a light line or band is seen crossing the bone in the position of the epiphyseal line, and a small spur of bone projects from the outside of both metatarsals. Lesions marked by arrows.

FIG. 15. 6 weeks later. Repair of the epiphyseal lesions. Note the general enlargement of the distal ends of the bones (lateral metatarsals), the loss of epiphyseal lines, and the thickening of the bones at the site of the lesions. Adjacent areas of the fourth metatarsals also appear to be slightly altered.

FIG. 16. 84 days. An unusually pronounced case of bone syphilis. There is necrosis of the right calcaneus associated with effusion into the surrounding tissues and swelling of the tarsus, foot, and leg. Necrosis of the left lateral metatarsal is also present, with effusion and swelling of the foot and tarsus, and a number of lesions may be seen in the phalanges of both feet (marked by arrows). Pathological fracture of several of these bones occurred a few days later.

FIGS. 17 to 21. Affections of the metatarsals and phalanges.

PLATE 71.

FIG. 17. 92 days. Periostitis and osteitis. Left foot: periostitis of all three phalanges of the fifth toe and of the second and third phalanges of the fourth toe. Right foot: periostitis of the terminal phalanges of the third toe and of the shaft of the lateral metatarsal; osteitis of the proximal and distal ends of the metatarsal (note absence of epiphyseal line) and of the distal end of the first phalanx of the fifth toe. Other shadows are from cutaneous lesions.

FIG. 18. 2 weeks later. Left foot: necrosis and buckling of the second phalanx of the fifth toe and loss of nail of the fourth toe. Right foot: necrosis and fracture of first phalanx of fifth toe.

FIG. 19. 1 year and 9 months. Marked permanent lesions of the phalanges.

PLATE 72.

FIGS. 20 and 21. Symmetrical affections of the lateral metatarsals and phalanges showing distributional tendencies.

FIG. 20. 85 days. Periosteal lesions on the lateral surface of the feet extending from the base of the metatarsals to the terminal phalanges. Other toes are slightly affected, notably the fourth toe of the left foot.

FIG. 21. 85 days. Serial lesions of the metatarsals and phalanges illustrating the bead-like effect produced in the rabbit's foot and various degrees of bone involvement associated with them.[2]

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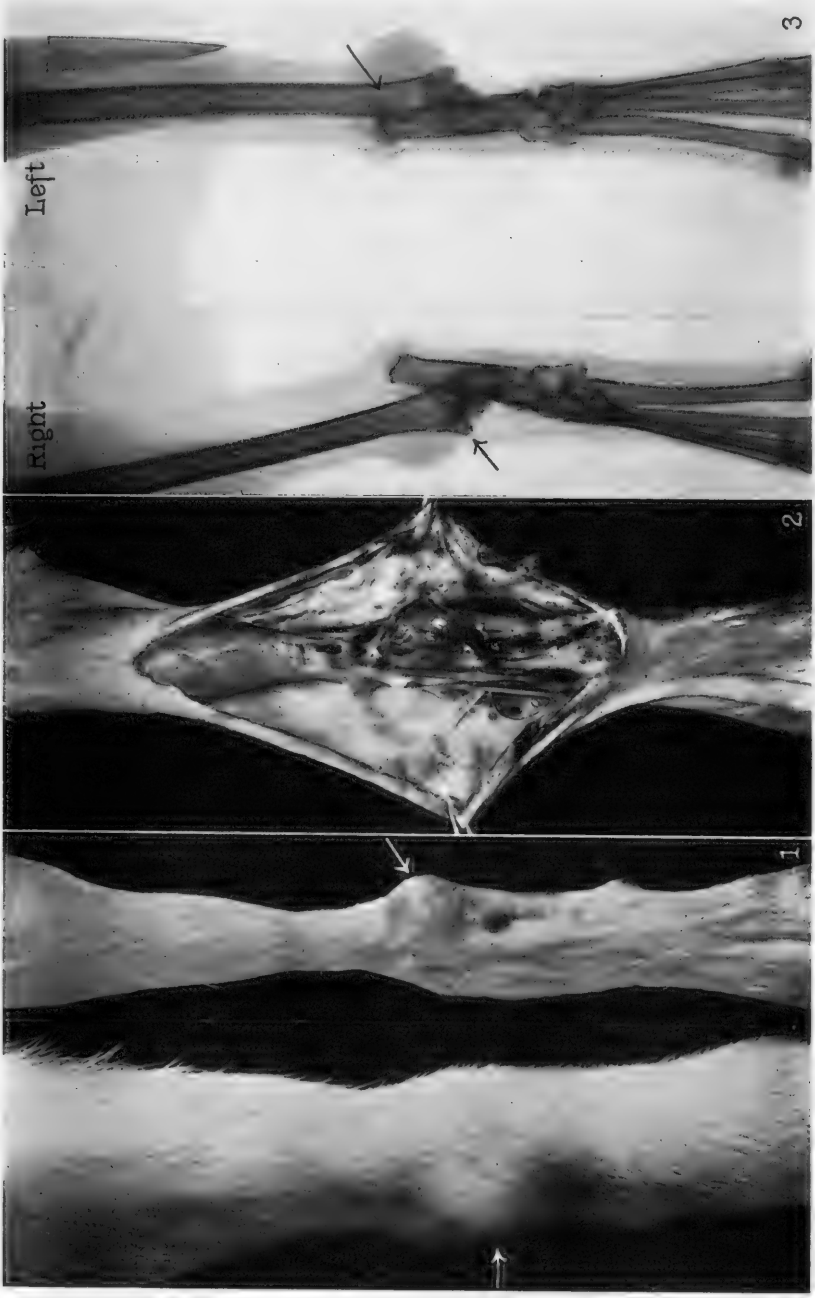
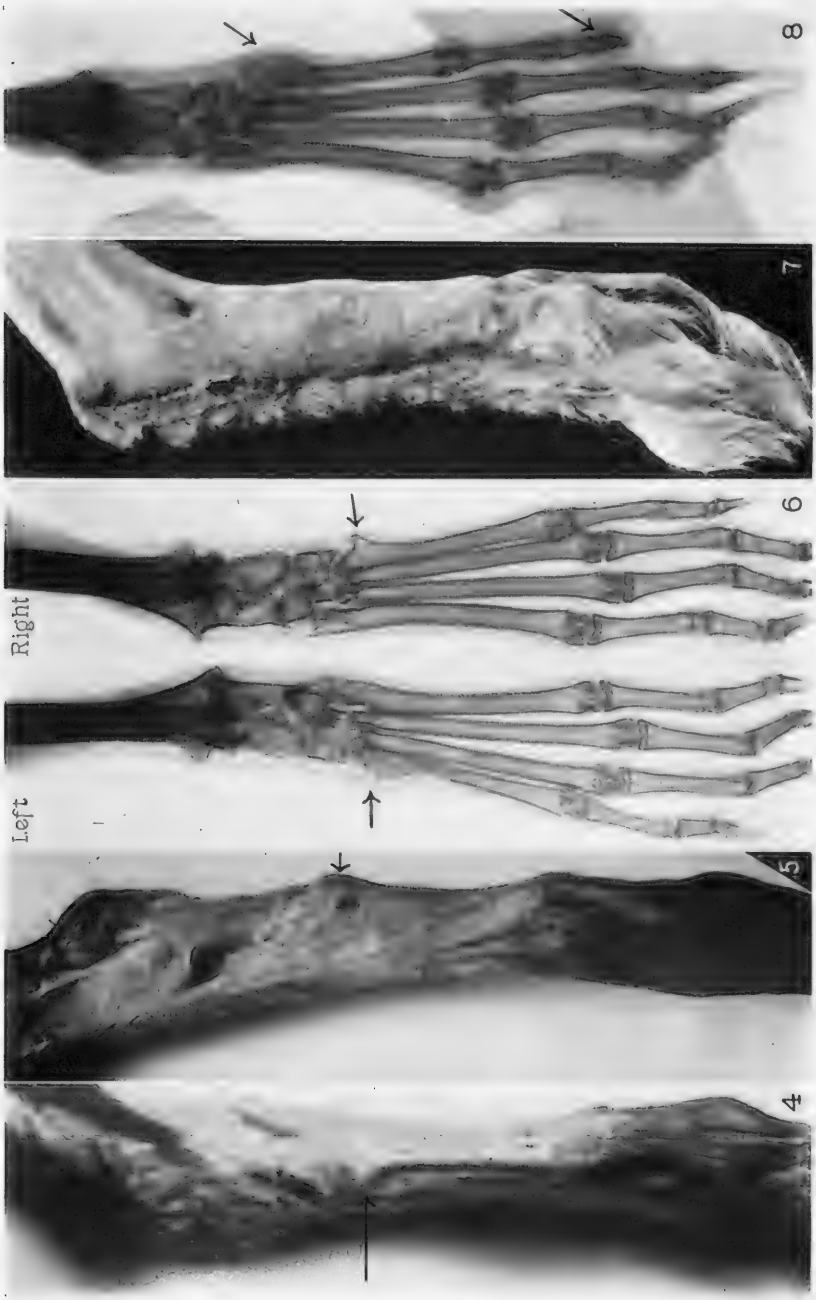
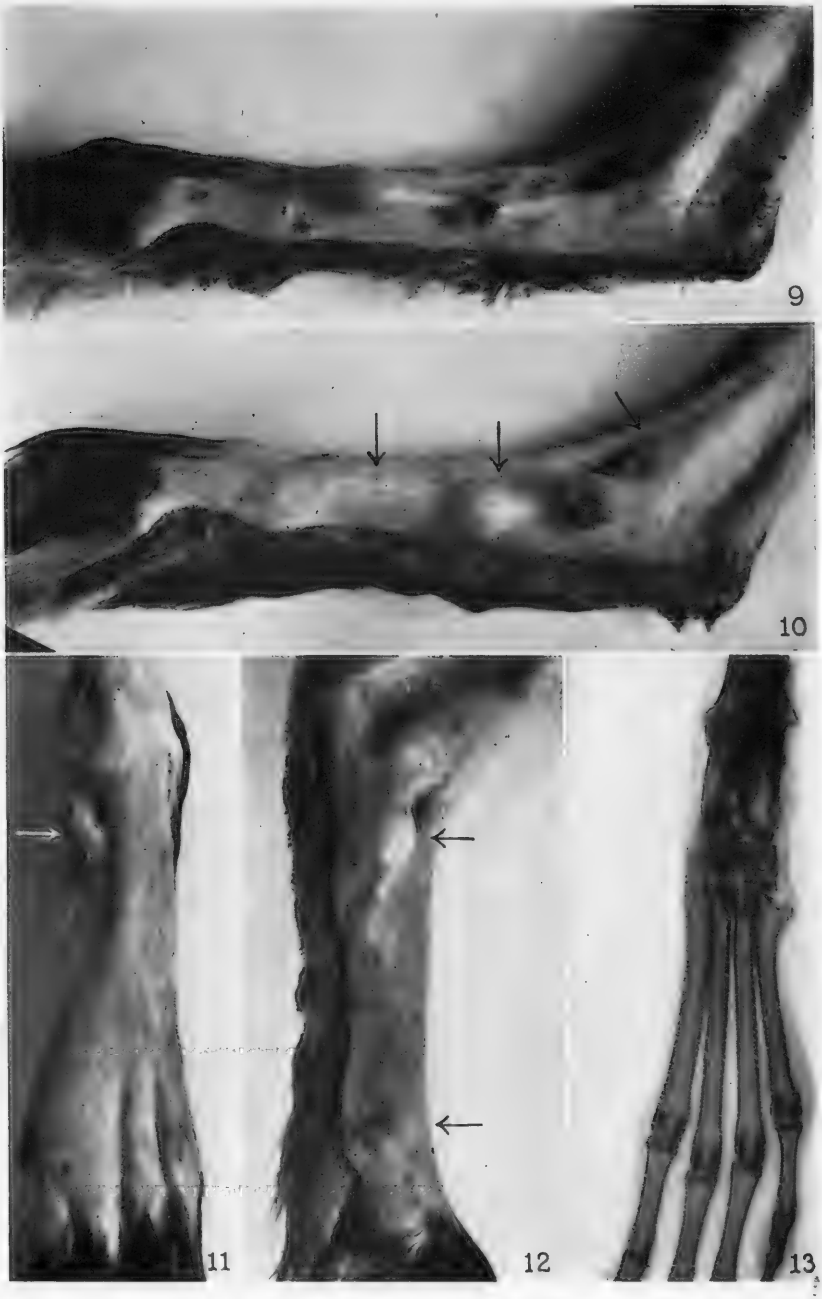
[2] See also Brown, Pearce, and Witherbee (1), Fig. 3.



(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)



(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)



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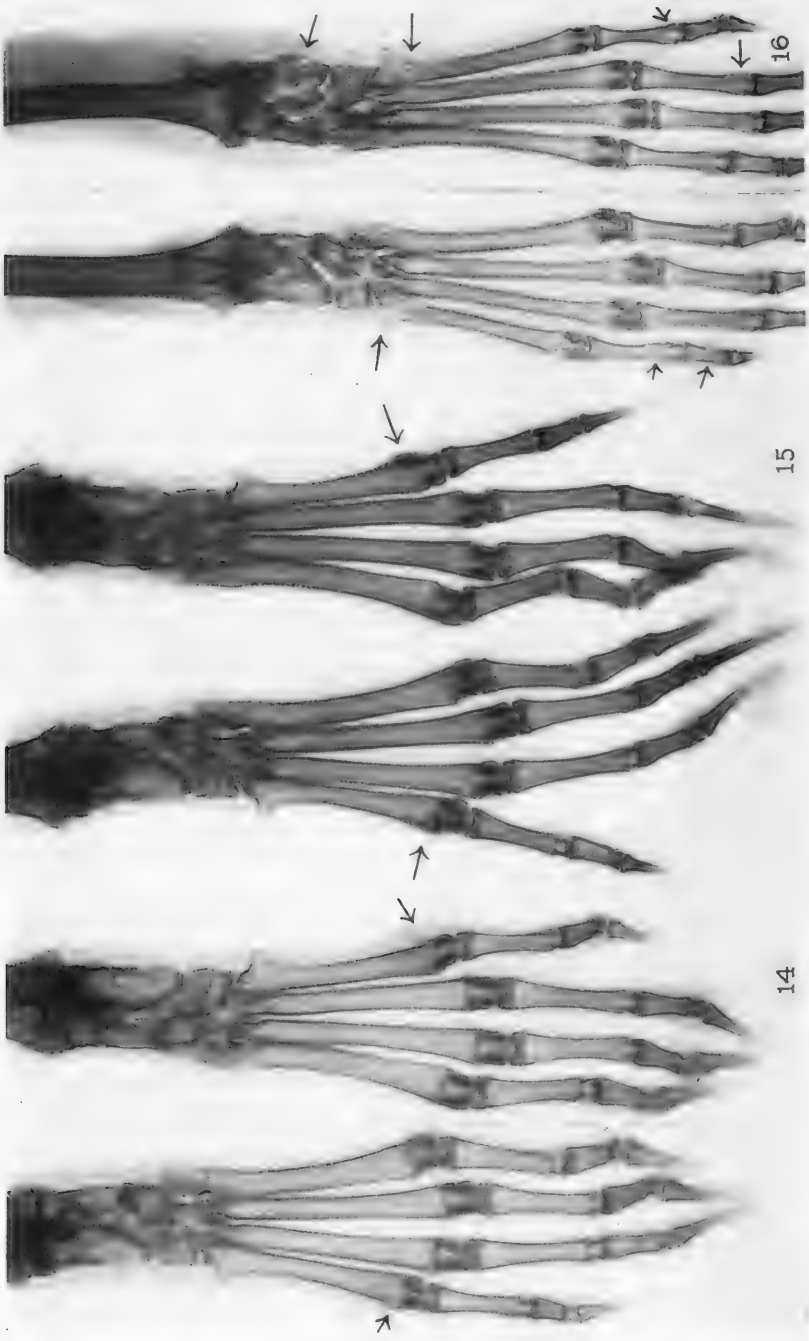

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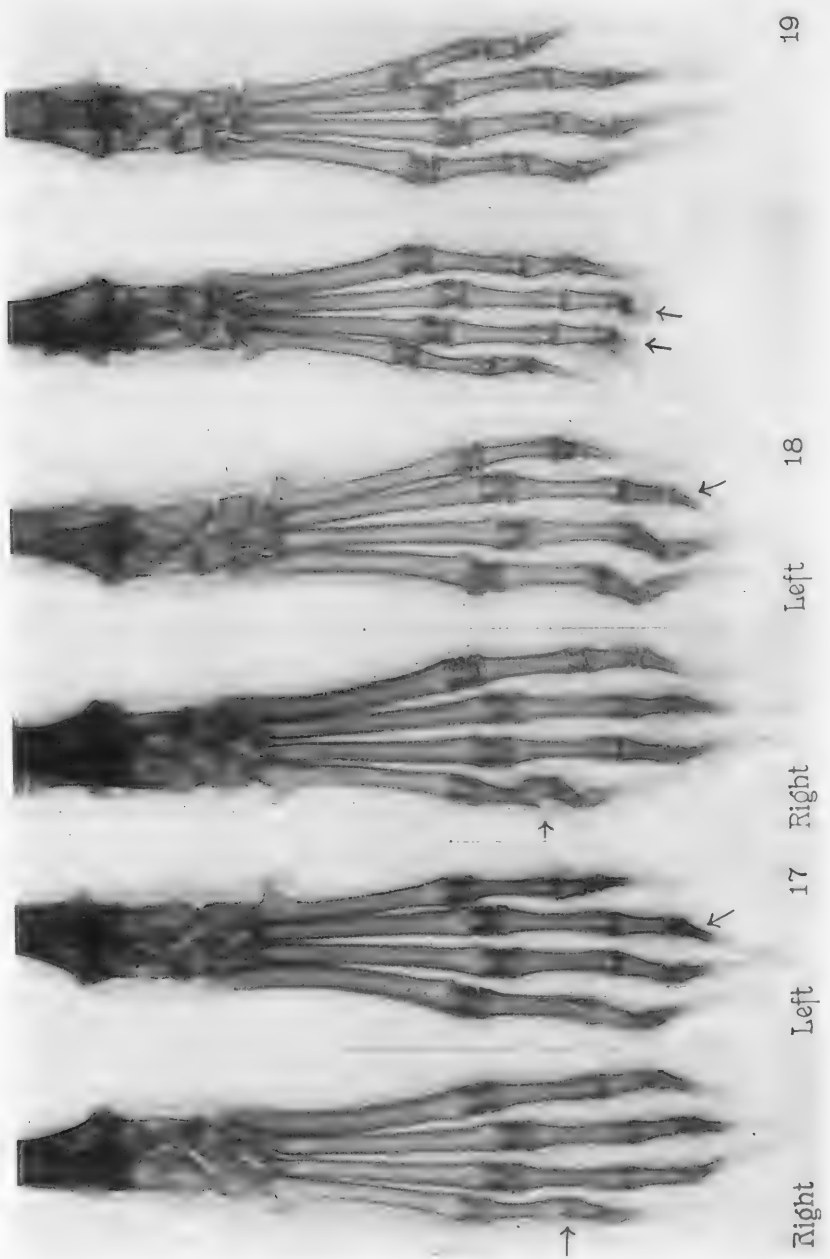
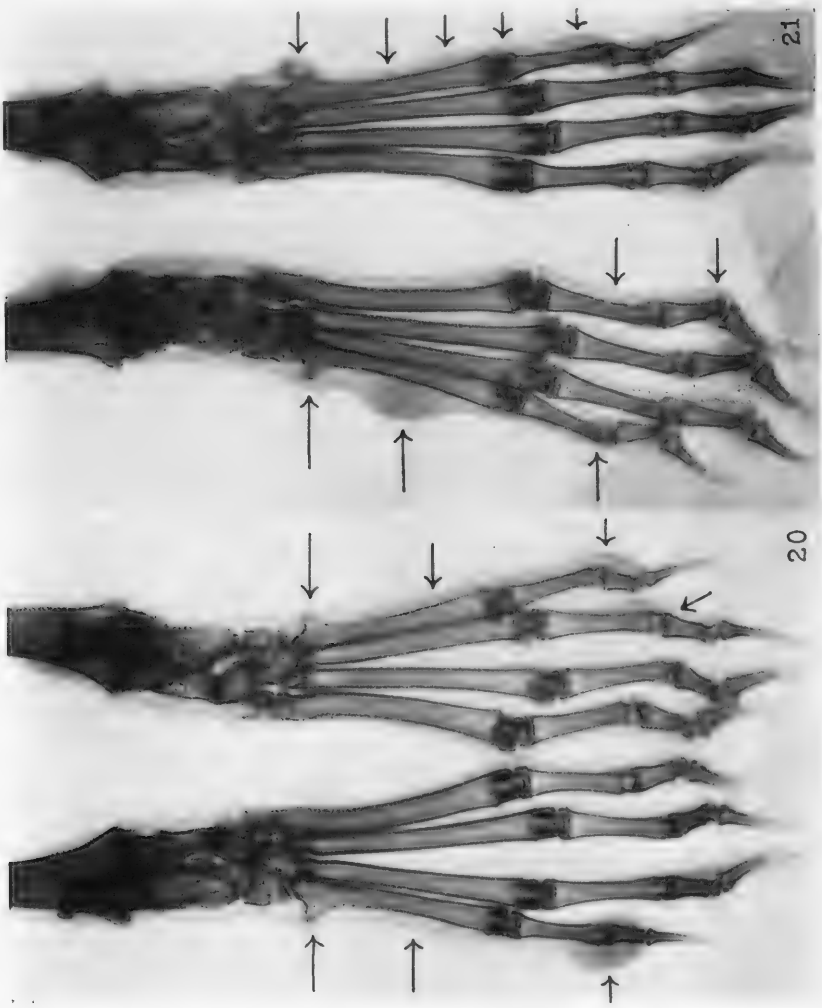


(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)



(Brown, Pearce, and Witherbee: Experimental syphilis in the rabbit. VI.)

A NEW STRAIN OF TRANSMISSIBLE LEUCEMIA IN FOWLS (STRAIN H).

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In previous reports (1915, 1918) the writer has described three types of leucemia in the fowl, the lymphatic, the myeloid, and the intravascular lymphoid[1] types. Reference has also been made to purely anemic forms, which appear to be closely related to the intravascular lymphoid type. Within one type the clinical picture of the disease presents many variations, which recall analogous instances in man. The association with neoplastic growths—lymphomata and myeloid tumors—is noteworthy. In previous experiments the author has demonstrated that the various types of leucemic disease may be produced by the same experimental strain. Hence the conclusion seems justified that all are produced by the same virus.

The study here reported, with a new strain, was undertaken in order to verify previous findings and to elucidate the histogenetic processes. It deals with general considerations relating to the strain, particularly the problem of virulence. The strain employed was derived from a hen with leucemia received on February 22, 1917, from Professor Carl Hansen.

Protocol 1.—Hen H. February 22, 1917. Color of comb pale. Hæmoglobin 20 per cent (Sahli). Blood films show well pronounced leucemia. Every microscopic field reveals myelocytes, but lymphoid cells predominate. These cells have dark, round nuclei and a scanty border of protoplasm. There is

[1] The term intravascular lymphoid leucemia is merely tentative. In this type, which is closely related to the pure anemic form, microscopic examination shows only intravascular deposits of lymphoid cells, which are probably erythroblasts in early stages. The type of disease must be regarded as a severe anemia with an excessive pathological regeneration. An article on this subject will appear shortly in *Folia hæmatologica.*

often marked basophilia. Mitotic divisions are numerous. There are few typical, small lymphocytes, polynuclear cells, and large mononuclear cells to be seen. Erythroblasts are found frequently. The oxidase reaction reveals blue granules in some myelocytes, but the granules in most of these cells are unstained. Erythrocytes 1,080,000; leucocytes 720,000. The differential count of leucocytes is given in Table I.

February 24, 1917. Animal killed.

TABLE I.

Differential Leucocyte Count of Hen H.

Cells.	Per cent.	No.
Polynuclears	2	14,400
Myelocytes	15	108,000
Large mononuclears	4	28,800
Lymphocytes	4	28,800
Lymphoid cells	75	540,000

Autopsy.—Liver.—Greatly enlarged; weight 156 gm. Color brownish, with numerous fine white spots on surface. These can barely be seen on the cut surface. *Spleen.*—Enormously enlarged; weight 40 gm. Bluish red in color. Cut surface quite uniform. *Kidneys.*—Brown with very fine white spots. Weight of right kidney 8 gm. *Heart.*—On anterior side shows a large white spot the size of a hemp-seed. *Lungs, stomach, intestines, thymus, bones, and skin.*—Show no pathological changes.

Microscopic Examination.—Liver.—Shows a number of medium sized periportal cell infiltrations, containing typical myelocytes. Non-granular cells are absent. The capillaries are markedly dilated and filled with large, round lymphoid cells and scanty myelocytes and erythrocytes. The cell masses produce complete injection of the capillary network. *Spleen.*—Only mere traces of follicles are seen. Pulp well filled with large lymphoid cells, which often show mitotic division. Perivascular layers of myelocytes are rarely encountered. *Bone marrow.*—The trabeculæ are partly atrophic and contain small dark cells. Some are of normal size and contain myelocytes. Fat cells are reduced in number. *Kidney.*—Interstitial infiltration with myelocytes in the superficial cortical layer. Capillaries often full of large lymphoid cells. *Heart.*—The white nodule consists of large cells, with oval nuclei and large masses of protoplasm without granules. Typical myelocytes are seen. *Intestine.*—In the deeper portions of the mucous membrane of the colon are found myelocytes in thin layers.

The case is one of typical leucosis with leucemia. The blood picture is characterized by the large number of leucocytes (myelocytes and

large lymphoid forms). The histological examination shows marked filling of the capillaries of various organs with leucocytes (leucostasis) and interstitial deposits of myelocytes in the liver, spleen, and kidneys. The case represents a mixture of the myeloid and the intravascular lymphoid types of disease.

Eight normal hens were inoculated into the wing vein with citrated blood from the original fowl. One of the inoculated birds developed leucosis. The inoculations were then continued through twelve generations. In all, 122 birds were inoculated. In 34 instances (28 per cent) the inoculations gave positive results. For comparison it may be mentioned that Ellermann and Bang (1908, *a*, *b*, 1909) had 39 per cent positive results from Strain A; Hirschfeld and Jacoby (1910), 44 per cent; Ellermann (1918), 22 per cent from Strain E; and Schmeisser, 33 per cent. One constantly finds a certain percentage of birds immune. The resistance is not, however, constant, as by repeated inoculations it may be possible to transmit the disease to fowls originally immune. Both these facts were again confirmed by the experiments made with Strain H.

Virulence of Inoculation Material.

In a previous article (Ellermann and Bang, 1909) it was pointed out that the number of takes in a single experiment may vary to a large extent. Hence it is necessary to employ a considerable number of birds in order to obtain positive results. If, for example, only five fowls are used, it often happens (in about 25 per cent of the experiments) that the outcome is negative notwithstanding the high degree of virulence possessed by the inoculation material. By using a larger number of fowls, the chances of obtaining positive results are increased, and with as many as twelve, transmission of the disease is practically certain. For the sake of economy, the writer was limited to eight fowls for each transmission experiment, with, as a result, somewhat lessened chances of a successful outcome. None of the fowls may acquire the disease, or, on the other hand, all may become infected. In the latter instance, we should not be justified in drawing the conclusion that the virus had a high degree of virulence. This variability in infectivity is shown in Table II. It would be advantageous if

it were possible to increase the number of infected animals, either by lowering the natural resistance of from 60 to 70 per cent of the fowls or by augmenting the virulence of the inoculation material. Attempts to lower the resistance by bleeding or hunger, made in previous experiments, were unsuccessful. An experiment of this character was undertaken with the present strain. Before inoculation of the leucosis virus an injection of tuberculin was given in eight fowls. The disease developed rapidly, appearing 2 or 3 weeks after

TABLE II.

Number of Fowls Infected with Strain H.

Generation.	No. of fowls inoculated.	No. of fowls infected.	Percentage infected.
1	8	1	13
2	20	7	35
3	8	5	63
4	16	2	13
5	16	3	19
6	8	1	13
7	8	3	38
8	6	1	17
9	8	4	50
10	8	4	50
11	8	2	25
12	8	1	13

inoculation, and the fowls died after a short interval—only 32 days. It has not been possible to repeat the experiment, but probably this result was accidental and can be explained in some other way.

A study of Table III, which gives the duration of illness in the successive generations of inoculated fowls, shows a progressive shortening of the period. At the beginning of the series the duration of the disease from the time of inoculation to death was 15 to 20 weeks, while at the end it was only 6 to 8 weeks. This shortening of the duration of the disease must be considered as attributable to increased virulence of the virus.[2]

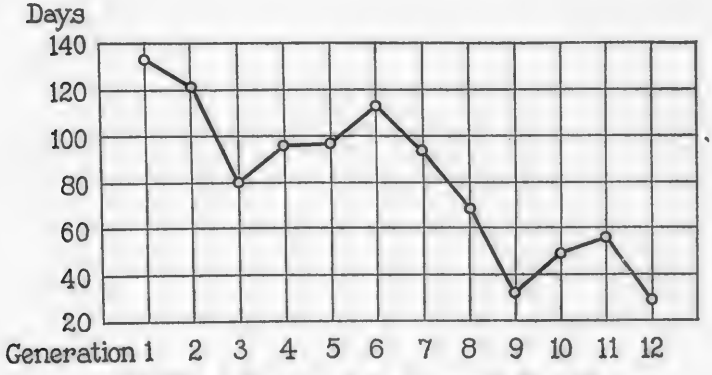
[2] Nothing can be stated concerning the true nature of the virulence, as different degrees of virulence may exist.

A graphic representation of the reduction in duration of the disease is given in Text-fig. 1. While the curve is not absolutely regular, the fall is distinct. The lack of regularity may be accounted for by

TABLE III.

Duration of the Disease with Strain H from the Time of Inoculation to Death.

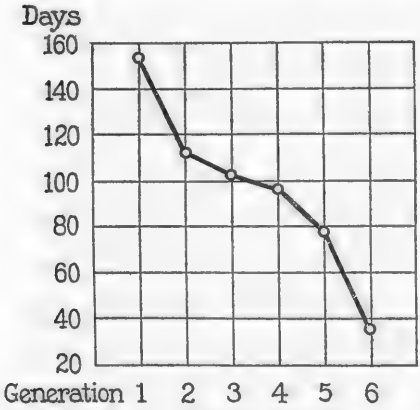
Generation.	Duration of disease.
	days
1	>133
2	121
3	80
4	96
5	97
6	113
7	94
8	69
9	32
10	49
11	56
12	29



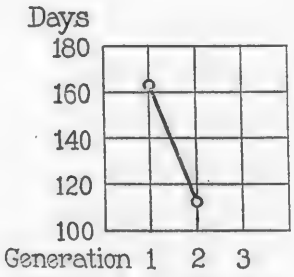
TEXT-FIG. 1. Duration of the disease with Strain H.

the irregularity in the sixth generation, caused, however, by only a single case. The fall in the ninth generation, in which the fowls had been treated with tuberculin previous to inoculation, is not very striking in comparison with the preceding part of the curve.

Text-figs. 2 and 3 are based on former experiments made with Strains E and D. It will be observed that these curves are similar to that of Text-fig. 1. The curve of Text-fig. 2 is very regular. By comparing the three curves it will be seen that the duration in the first generation is about the same for the three strains. It must be added that the quantity of material inoculated varied somewhat in the different experiments. As a rule, however, the amount consisted of a blood suspension corresponding to 2 drops of blood of the sick fowl. Sometimes 1 cc. of a centrifuged tissue emulsion was used. A study of this point shows that the variations in the curves



TEXT-FIG. 2. Duration of the disease with Strain E.



TEXT-FIG. 3. Duration of the disease with Strain D.

cannot be explained on the basis of the quantity inoculated. Hence it is possible to increase the virulence of the virus of leucosis by passage. This finding suggests similar results obtained with another filterable virus; namely, that of hydrophobia. By inoculating the street virus of a spontaneous case the incubation period decreases with each generation, until it reaches a fixed point. The increased virulence of the virus of leucosis used in the fowl is shown by a shortening in the duration of the illness. It is noteworthy, however, that there is no coincident rise in the number of successful infections. It appears doubtful whether any increase in the number of infections can be obtained through increased virulence of the virus.

The shortening of the duration of the disease with continued passage was partly overlooked in former experiments, and partly explained in another way. It was believed that the duration was determined by the type of the disease; that is, that the intravascular lymphoid cases had a shorter duration than the lymphatic cases. This viewpoint cannot, however, now be maintained. The main factor, apparently, is the position of the individual case in the series of generations. Hirschfeld and Jacoby (1910) were correct in their assertion that the length of the incubation period is an indicator of the degree of virulence. With Strain A of Ellermann and Bang, which had required an incubation period of about 8 weeks in the first generation, they observed an incubation period of 6 weeks at the beginning of their series, which later decreased to 4 weeks. The conclusions of Hirschfeld and Jacoby seem, however, to be based on a very small series of observations, and the use of the incubation period as an indicator of virulence has disadvantages. For instance, the time of onset of the disease cannot be established accurately if the onset is gradual or the course intermittent. In the lymphatic cases we cannot use the term incubation period, inasmuch as anemia and blood changes are usually absent.

Types of Disease.—It was observed with Strains D and E that the type of the resulting disease appeared to vary. Strain D, which originated from an intravascular lymphoid case, produced in the first generation a myeloid, and in the second generation a lymphatic case. From Strain E, originating from a lymphatic case, a series of lymphatic and intravascular cases, and also a few myeloid cases, resulted.

Table IV shows the results of experiments with Strain H, which was obtained from a case of mixed myeloid-intravascular character. In the first eleven generations we find myeloid, intravascular lymphoid, and anemic cases, while in the twelfth generation a lymphatic case suddenly appears. Table V gives the number of cases of each type.

The observations made on Strain H confirm the finding of a variation in the type of disease. The clinical pictures are varied, and intermediary forms difficult to classify are also present. A change from the myeloid to the lymphatic type, or the reverse, is rare. Caution

is necessary in diagnosing cases, and the possibility of occasional spontaneous cases must be kept in mind. Each strain seems to have a tendency to produce a certain type of disease. Strain E, for instance,

TABLE IV.

Types of Disease Obtained with Strain H.

Generation.	Types of cases.
1	Intravascular (No. 1).
2	Anemic (No. 2); intravascular (Nos. 3, 4, 5, and 7); myeloid (No. 6); myeloid and intravascular (No. 8).
3	Intravascular (Nos. 9 and 14); myeloid (Nos. 10 and 11); anemic (Nos. 12 and 13).
4	Anemic (Nos. 15 and 19); myeloid (Nos. 16 and 17); intravascular (No. 18).
5	Intravascular (Nos. 20, 21, and 22).
6	" (No. 23).
7	" (Nos. 24 and 25); myeloid (No. 26).
8	" (" 27 " 28).
9	" (" 29, 30, and 32); myeloid (No. 31).
10	" (" 33 and 35); myeloid (Nos. 34 and 36).
11	" (No. 37); myeloid (No. 38).
12	Lymphatic (No. 39).

TABLE V.

Number of Cases of Different Types Produced by Strain H.

Type of case.	No.
Intravascular and anemic	27
Myeloid (leucemic)	9
" (aleucemic)	2
Lymphatic	1
Total	39

produces mainly intravascular and lymphatic, and Strain H chiefly intravascular and myeloid cases. It appears, then, that the same virus at times produces a myelotrophic and at other times a lymphotrophic effect. Further investigation is needed to elucidate this point.

Serological Properties of Different Types.

An attempt to find isohemolysins or isoagglutinins gave negative results. On the other hand, it was found that the hemolytic power of the serum against rabbit erythrocytes was diminished in a fowl showing anemia. While normal fowl serum dissolved rabbit erythrocytes in about 6 minutes, it required a considerably longer period to produce hemolysis with serum from the anemic fowl. The conditions of the experiment were identical in both cases.

TABLE VI.

Hemolysis in Cases of Intravascular Lymphoid Leucosis.

Fowl No.	Date.	Hemoglobin (corrected).	Length of time required for hemolysis.	Length of time required for hemolysis in normal serum control.
	1917	*per cent*	*min.*	*min.*
5	Sept. 25	30	15	5
	Oct. 7	42	14	8
7	" 1	75	16	8
	" 17	58	12	5
3	" 3	33	20	8
	" 17	25	10	5
13	" 27	25	10	6

TABLE VII.

Hemolysis in Cases of Myeloid Leucosis.

Fowl No.	Date.	Hemoglobin (corrected).	Length of time required for hemolysis.	Length of time required for hemolysis in normal serum control.
	1917	*per cent*	*min.*	*min.*
8	Oct. 4	42	10	8
6	Nov. 1	33	6	6
17	" 3	33	5	7

The technique employed was that described below. Rabbit blood was mixed with one-tenth of its volume of a 10 per cent solution of sodium citrate. After centrifugation, the blood corpuscles were used directly, without washing, for making a 3 per cent suspension in 0.9 per cent solution of sodium chloride. The fowl serum was prepared by keeping the blood in a cool room for 24 hours. 0.1 cc. of blood suspension was

added to 0.2 cc. of the active serum by means of a capillary pipette in such a manner as to produce an instantaneous mixture.[3] Tables VI and VII give the results of these experiments. The former comprises four cases of intravascular lymphoid leucosis. It will be seen that in comparison with normal serum it required twice as long to produce hemolysis in these cases. It can scarcely be asserted that the anemia is the causative factor, as the percentage of hemoglobin was not exceedingly low in any case. In one instance (the first experiment with No. 7) there was no reduction of hemoglobin, the percentage being the same as before inoculation.

TABLE VIII.

Titrations to Test Hemolytic Power.

Fowl No.	Date.	Titer.	Titer of normal control.
	1917		
3	Oct. 17	1:4	1:8
7	" 17	1:4	1:8
13	" 27	1:4	1:8

The findings are quite different in Table VII, which shows two cases (Nos. 6 and 17) of typical myeloid leucemia, and a mixed case (No. 8) characterized by a myeloid subleucemic blood picture and myelosis combined with intravascular leucostasis of the organs. In the mixed case there appears to be a slight retardation of hemolysis, but in the two cases of myeloid leucemia there is no diminution of hemolytic power. On the contrary, the hemolysis with serum from Fowl 17 is more rapid that that of the control. In view of these observations it is suggested that the power to produce hemolysis may depend upon the presence of myeloid tissue. This tissue, which was negligible in the first group of cases, was increased in the second group.

Although the experiments are few in number the phenomenon of a retardation in hemolysis appears to be a well established fact. As the determination of the moment of complete hemolysis depends to a certain extent on the personal element, as a matter of precaution titrations were done to test the hemolytic power in some cases in

[3] Agglutination of the erythrocytes invalidates the experiment.

Table VI. A series of dilutions (1:1, 1:2, 1:4....1:64) was made of active serum. After the components had been mixed as described above, they were kept at a temperature of 60°C. for 2½ hours and were shaken and placed in a cool room until the following day. The results are shown in Table VIII.

The titer, that is the dilution at which complete hemolysis is obtained, is shown to be in the case of the leucemic fowls about half that of the normal controls. This corresponds to the retardation of hemolysis which has been described.

Further Experiments.

Filtration of Inoculation Material.—Previous experiments by Ellermann and Bang (1908, *a*, *b*, 1909) and Ellermann (1913, 1918) have proved that the virus is filterable, and that intact cells are not necessary in order to transmit the disease. As other investigators have had small success with positive filtration experiments, the results of an experiment with Strain H are given below.

15 gm. of the organs of Hen 1 (first experimental generation) were mixed with 0.9 per cent salt solution. This emulsion was filtered through cotton and diluted to make 100 cc. The fluid was passed through a Berkefeld filter No. 12. The filtrate was completely clear and gave no growth on agar or broth, while the control from the unfiltered emulsion gave rise to a rich growth. Ten normal birds (Nos. 7, 8, 18, and 40 to 46) were inoculated with the filtrate, each receiving 1 cc. Two fowls (Nos. 7 and 8) became infected. The incubation period and the total duration of the disease did not differ from those of the controls inoculated with unfiltered material.

Immunization Experiments.—Since experiments conducted by Ellermann and Bang and Hirschfeld and Jacoby have shown that subcutaneous inoculation does not infect fowls, it was decided to attempt immunization in this manner. Eight normal hens (Nos. 14 and 47 to 53) were inoculated subcutaneously with an emulsion of leucemic organs (No. 8), each hen receiving 2 cc. 3 weeks later the birds received an intravenous injection of emulsion from another infected hen (No. 5). One bird died 2½ months later. The postmortem and microscopic examinations showed a well marked leucosis. Hence it ap-

peared that the subcutaneous injection of virulent material had not produced immunity.

Inoculation of Citrated Blood Plasma.—These experiments were made in order to determine whether the virus is localized in the blood corpuscles or in the plasma or in both. 30 drops of blood were placed in a mixture of a 0.9 per cent salt solution and 10 per cent sodium citrate. After vigorous centrifugation the upper portion of the clear fluid was taken up in a sterile syringe and injected into eight normal hens (Nos. 15 and 54 to 60); eight other hens (Nos. 16 and 61 to 67) received injections of the blood cells. It was found that one fowl of each group (Nos. 15 and 16) contracted the disease.

In the experiments undertaken with another series of birds (Nos. 20 and 68 to 82) only one positive result was obtained with blood corpuscles, none with the plasma series. The objection might be raised that the separation of blood corpuscles and plasma cannot be considered effective, although this procedure is preferable to filtration, which injures the cells. The experiments seem to suggest that the virus is contained in the blood plasma. Nothing has been determined concerning the relation of the blood corpuscles to the virus. As it would hardly seem possible to remove a virus adherent to the surface of the cells, this has not been tried.

Inoculation with Human Material.—This experiment has no relation to the strain of fowl leucosis. It was undertaken solely to determine whether the disease in man and that in the fowl are caused by the same agent. Von Wiczowsky reports positive results in an experiment of this kind. On the other hand, experiments made by Hirschfeld and Jacoby (1909) and by Wechselmann and Hirschfeld were negative. *A priori*, the chances of a successful experiment seemed small, as attempts made to infect other birds of closely related species by means of fowl material have always failed. 2 cc. of blood taken from a patient, aged 43 years, suffering from myeloid leucemia in Bispebjorg Hospital, were mixed with a sodium citrate solution. 2 hours later this blood suspension was injected into eight normal hens (Nos. 83 to 90). The fowls were observed during a period of 6 months, but no symptoms of disease were discovered. Hence it seems doubtful whether the experiment of von Wiczowsky can be accepted. It is probable that every species has its own particular form of leucemia which cannot be transmitted to other species.

SUMMARY.

1. A new strain of fowl leucosis has been transmitted through twelve generations of fowls.

2. An increase in virulence was observed during its passage. This was shown in a shortening of the interval between inoculation and death. The increase in virulence does not affect the number of successful inoculations, which remains approximately constant in from 20 to 40 per cent of the birds employed.

3. As with former strains, the disease manifests itself in various forms; *i.e.*, myeloid and intravascular lymphoid types. A single lymphatic case was observed.

4. In several intravascular cases a diminution in the hemolytic power of the serum was established. This phenomenon was absent in a number of myeloid cases.

5. Active immunization cannot be produced by means of the subcutaneous injection of virulent material.

6. The finding of previous experiments that the virus is filterable has been confirmed.

7. The inoculation of human leucemic material into fowls gave negative results.

BIBLIOGRAPHY.

Ellermann, V., and Bang, O., Experimentelle Leukämie bei Hühnern, *Centr. Bakt., Ite Abt., Orig.*, 1908, *a*, xlvi, 4.

Ellermann, V., and Bang, O., Experimentelle Leukæmie bei Hühnern, *Centr. Bakt., Ite Abt., Orig.*, 1908, *b*, xlvi, 595.

Ellermann, V., and Bang, O., Experimentelle Leukämie bei Hühnern. II, *Z. Hyg. u. Infectionskrankh.*, 1909, lxiii, 231.

Ellermann, V., Untersuchungen über das Virus der Hühnerleukämie, *Z. klin. Med.*, 1913, lxxix, 43.

Ellermann, V., Untersuchungen über die übertragbare Hühnerleukose, *Berl. klin. Woch.*, 1915, lii, 794.

Ellermann, Vergleichende Leukosestudien, *Virchows Arch. path. Anat.*, 1918, ccxxv, 115.

Ellermann, V., Die übertragbare Hühnerleukose, Berlin, 1918.

Hirschfeld, H., and Jacoby, M., Zur Kenntnis der übertragbaren Hühnerleukämie, *Berl. klin. Woch.*, 1909, xlvi, 159.

Hirschfeld, H., and Jacoby, M., Uebertragungsversuche mit Hühnerleukämie, *Z. klin. Med.*, 1910, lxix, 107.

Hirschfeld, H., and Jacoby, M., Uebertragbare Hühnerleukämie und ihre Unabhängigkeit von der Hühnertuberkulose, *Z. klin. Med.*, 1912, lxxv, 501.

Schmeisser, H. C., Spontaneous and experimental leukemia of the fowl, *J. Exp. Med.*, 1915, xxii, 820.

Wechselmann and Hirschfeld, cited by Hirschfeld and Jacoby (1909).

von Wiczowsky, J., Beitrag zur Lehre über die Leukämie, *Wien. klin. Woch.*, 1913, xxvi, 569.